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The effects of limestone particle size on bone health and performance of pullets and hens in conventional cage and alternative housing systems

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THE EFFECTS OF LIMESTONE PARTICLE SIZE ON BONE HEALTH AND PERFORMANCE OF PULLETS AND HENS IN CONVENTIONAL CAGE AND ALTERNATIVE HOUSING SYSTEMS

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THE EFFECTS OF LIMESTONE PARTICLE SIZE ON BONE HEALTH AND PERFORMANCE OF PULLETS AND HENS IN CONVENTIONAL CAGE AND ALTERNATIVE HOUSING SYSTEMS

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One of the major welfare issues in cage-free housing systems is bone fracture. The goal of this research was to improve bone health in layers by building better pullet skeletons and to investigate limestone particle size (LPS) effects on bone health in conventional cages and cage-free housing systems. Study 1 was a preliminary study that compared conventional cages with litter floor pens in terms of performance, bone health, and eggshell quality from 33 to 47 wk of age. Caged hens had higher egg production and eggshell strength, and improved feed efficiency, but lower tibia bone mineral content compared to floor housed hens. Study 2 examined the effects of LPS fed from 7 to 17 wk of age on performance and bone health in conventional cage and aviary systems. The use of a limestone blend of fine and large particles (0.879 mm; LPS-Blend) rather than a fine limestone (0.431 mm; LPS-Fine) increased tibia bone mineral density (BMD) and alleviated incidence of curved keel bones at the end of the pullet phase. Study 3 investigated the subsequent effects of LPS (Study 2) during the layer phase. The LPS-Blend increased eggshell weight and alleviated keel bone indentations in the middle and end of the lay cycle (Study 3). Study 4 examined the effects of LPS fed from 9 to 17 wk of age on pullet and hen performance, bone health, and eggshell quality in deep litter systems. Hens fed LPS-Blend during the pullet phase had greater tibia BMD at onset of egg production and higher overall eggshell strength. Study 5 evaluated the effect of two

layer strains on nest and perch use in aviary systems. White Leghorn hens had greater usage of perch and nest and preferred elevated tiers compared to Brown hens. In conclusion, the provision of LPS-Blend rather than LPS-Fine during the pullet phase improved bone mineralization at the onset of egg production and eggshell quality. Although White Leghorn hens had greater usage of resources in aviary systems; they had higher potential risk of bone fractures.

DEDICATION

This dissertation is dedicated to my beloved parents and husband.

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CHAPTER 1. LITTERATURE REVIEW

1.1 INTRODUCTION

Conventional cage systems have been the most popular housing systems for laying hens in the United States for more than fifty years. However, recently, hen welfare has become a relevant global issue for consumers, producers and policy makers. The ban of conventional battery cages around the world are forcing egg producers to switch to alternative housing systems. Housing types of current production systems are classified into conventional cage, enriched cage and cage-free systems. The latter is further subdivided into deep litter or barn, aviary, free-range systems (Fröhlich et al., 2012).

In the European Union (EU), the Directive 1999/74/EC phased out conventional cages for hens by January 1st, 2012. Enriched colony cage system has been the most widely adopted system in Europe which contains 20 to 60 hens at stocking density mandated by the EU (Mench et al., 2011). Some other European countries such as Switzerland and Sweden prohibited the utilization of conventional cages even before that deadline. In 2002, the United Egg Producers (UEP), representing nearly 90% of U.S. egg producers, launched the UEP Certified Program, which requires increasing stocking space for laying hens from 48 to 67–86 square inches per bird (United Egg Producers, 2014). In 2008, California passed the Prevention of Farm Animal Cruelty Act, which requires that by 2015, cages will be large enough for a hen to stand up, turn around, and flap its wings without touching the side of the cage or another laying hen. Michigan passed a similar law in 2009 forbidding conventional cages by 2019. Similar regulations are being debated in other states, including Ohio and Oregon. In 2010, Ohio agreed to place a moratorium on the construction of houses with new conventional cages as part of

an agreement to stop a ballot initiative (Greene and Cowan, 2013). These propositions will likely impact interstate marketing of table eggs.

Some sectors of U.S. egg operations are facing the challenge of being forced to switch from conventional cage housing system to enriched caged housing system, while others may consider alternative housing systems for product diversity to satisfy a growing niche market for cage-free eggs (Xin et al., 2012). Sales of eggs that are differentiated from conventional eggs by nutrient content or the circumstances of raising hens have increased steadily and accounted for nearly 16% of the entire egg market in 2005 (Chang et al., 2010). In particular, organic egg sales have grown rapidly at an average annual rate of 19% from 2000 to 2005 (Oberholtzer et al., 2006). A more recent survey in the U.S. indicated that 85 % of respondents were willing to pay a premium to improve hen welfare attributes, particularly cage-free environment with the highest average premium of $ 0.49 per dozen (Heng et al., 2013).

Even though cage-free housing systems improve some welfare aspects, they also increase feed, labor and housing costs (Sumner et al., 2010; Anderson et al., 2014). In addition, hens raised in alternative housing systems can have greater mortality rate than caged hens due to cannibalism and greater risk of diseases and parasitic infections from exposure to their own droppings (Weeks, 2012). Bone fracture is not a major cause of high mortality in alternative housing systems but its higher incidence in those production systems makes it a serious welfare and production issue. Eggs laid outside of the nest box are also an economic problem in non-cage systems which lead to more uncollectable, downgraded or unmarketable eggs. Higher incidence of floor eggs increases labor costs because those eggs need to be collected by hand and could reduce hen-day egg

production because of increased number of broken eggs (Sumner et al., 2010). In addition, Xin et al. (2011) suggested that more energy resources will be needed during winter for some cage-free and alternative systems because of lower stocking densities.

Thus, the implementation of alternative housing systems will result in higher total egg production costs. It is going to be even more important to improve production efficiency in these cage-free housing systems while maintaining hen welfare and an environment that promotes normal hen behavior. The study of nutritional strategies to reduce bone fractures in alternative housing systems will provide valuable information for the egg industry in light of the forthcoming changes in U.S. housing systems.

1.2 WELFARE AND PRODUCTION CONCERNS IN ALTERNATIVE HOUSING SYSTEMS

Skeletal problems

Osteoporosis, a decrease in the amount of fully mineralized structural bone, has been a major skeletal problem in the egg industry for the past fifty years (Whitehead, 2000). Increased activity in alternative housing systems improved bone strength (Jendral et al., 2008) but there was still a high incidence of bone fractures in those systems. The highest incidence of bone fractures has been observed in free-range systems provided with aerial perches and multi-tier aviary systems whereas the lowest incidences have been observed in conventional and furnished cages (Wilkins et al., 2004).

The most frequently damaged bone was the humerus in conventional cages (Gregory et al., 1990) and the keel and furcula (fork-like bone formed by the fusion of two clavicles) in housing systems with perches (Gregory et al., 1990; Wilkins et al., 2004). Because of the thin structure and anatomically exposed location of a layer's keel

bone, keel bone integrity could be negatively affected in alternative housing systems (Pickel et al., 2011). Keel bone damage have been associated with trauma (Fleming et al., 2004) because of collision with equipment inside the house, bad landings (Gregory et al., 1990), and long term pressure on the keel during roosting (Pickel et al., 2011).

Scholz (2007) showed that more than 70 % of hens raised in alternative housing systems with severe and moderate keel bone deformations had new woven bone and fracture callus material in a histological examination indicating a traumatic origin. Less than half of hens (41 %) with slight keel bone deformation (S-shaped deviation) did not have histological signs of bone fracture (Scholz, 2007). The author suggested that this type of deformation could be caused by extended pressure loading while perching rather than short-duration trauma.

It is generally agreed that calcium, phosphorus, and vitamin D play major roles in bone integrity (De vries et al., 2010; Fleming et al., 2008; Leeson and Summers, 1988; Rath et al., 2000); however, the effect of nutrition on keel bone deformities has not been established with modern layer strains. Past research indicated that deficiency of vitamin D increased incidence of crooked (S-shaped) keel bones for young caged pullets (Jonson and Smith, 1944; Warren, 1937). Perhaps nutrition could also be partially involved in the development of keel bone deformities. Further studies in this matter could be useful to find ways to alleviate keel bone deformities and fractures.

Another potential source of bone damage in laying hens, particularly in alternative systems, may be wing flapping itself. The pectoral girdle of birds might not be able to withstand all the stresses developed by the flight muscles (the pectoralis and the supracoracoideus) during both flapping and flight. The presence of medullary bone tissue

in the keel bone during the layer period (Whitehead, 2004) may change the biomechanical properties of the keel bone and the onset of osteoporosis may mean that the keel can no longer withstand these actions without incurring damage (Sandilands et al., 2009).

Mislaid eggs

Nests in the commercial poultry industry are usually widely accepted by laying hens and the number of mislaid eggs is generally far below 10%. However, at the start of lay, the number of mislaid eggs may range from 15% to over 80% (Huber-Eicher, 2004). Floor eggs have a great economic impact in alternative housing systems because those eggs must be collected by hand. Also, mislaid eggs may be eaten by the hens or become cracked, dirty, and/or contaminated with bacteria in the litter.

In floor systems, most eggs are laid in only a few of the available nests, usually nests in the corners or at the end of a row. This strong preference for a limited number of nests results in a reduction of available nests of choice, increasing not only aggression between the hens (Nicol et al., 1999), but also the number of mislaid eggs. There is a need in terms of animal welfare and economy to find ways to make nests away from the corners and the ends of nest rows more attractive to distribute the hens more evenly. Attractiveness of nest boxes could be influenced by nest color, light intensity, nesting material, height of perches, and degree of seclusion. Appleby et al. (1984) indicated that White Leghorn strain exhibited the expected preference for dark nests while a strain derived from Rhode Island Reds was more likely to lay in light colored nests. Regarding nest floor lining, hens preferred peat or artificial turf rather than plastic mesh (Struelens et al., 2005). The lowering of perches in furnished cages reduced broken outside-nest

eggs; however, it disturbed perching behavior during the light period (Tuyttens et al., 2013).

Some experiments in floor pens or colony cages have showed that Brown and White hens had different degree of nest usage and incidence of floor eggs (Abrahamsson and Tauson, 1995; Faure and Jones, 1982; Silversides et al., 2012). However, there is no study of strain effect on nest usage in aviary systems.

Perch utilization

Hens are highly motivated to use perches and will use them to reach resources, to roost at night, and to escape unwanted attention from other birds (Sandilands et al., 2009). Enneking et al. (2012) reported that caged pullets start to perch as early as 2 wk of age, though perching was rare this early. Perch usage increased with age, peaking at 12 wk of age, and this level of perching activity was maintained until the end of the pullet rearing phase. Early use of perches has also been reported for pullets reared in non-cage systems. For example, 91 % of pullets given access to roosts at 4 wk of age in a littered floor pen began perching within a week, whereas older pullets exposed later in life to perches (8 to 20 wk of age) did not show similar rates of perching until 37 wk of age (Appleby and Duncan, 1989). Perch frequency could have an economic impact as it is linked to the rate of floor eggs. For instance, there were four times more hens laying their first egg on the floor when they were reared without perches than those provided with perches during the pullet rearing phase (Appleby et al., 1986).

Perch behavior is affected by strain, with Brown hens having the lowest frequency of roosting (Faure and Jones, 1982; Silversides et al., 2012; Abrahamsson and Tauson, 1995). Silversides et al. (2012) observed that more White hens (76.3 %) than

Brown hens (6.8 %) roosted on perches before light went off in floor pens. Moinard et al. (2004) suggested that perch use of Brown hens might be compromised by wing loading calculated by body weight to wing area ratio. The wing loading of Lohmann Brown layers is approximately twice as great as the red jungle fowl, its ancestor; primarily due to greater BW but similar wing areas (Moinard et al., 2004).

In addition, some researchers indicated that perches reduce the incidences of feather pecking and cannibalism in cage-free systems. In a large scale Swedish survey that comprised a sample of 59 aviary flocks with 120,385 hens, the access to perches from 4 wk of age decreased the prevalence of cloacal cannibalism during the layer phase (Gunnarsson et al., 1999). Also, among ten management practices, the access of elevated perches (35 cm or more above the floor) and high density (more than 10 birds per m^2) were the factors that contributed significantly and strongly to the prevalence of feather pecking in a multinomial analysis (Huber- Eicher and Audigé, 1999). It is suggested that hens used perches as a refuge to avoid aggression such as feather pecking. The height of the perch may be of particular importance. There was less vent pecking and overall feather loss with perches 70 cm above the floor compared to 45 cm (Wechsler and Huber-Eicher, 1998) probably because hens standing on the floor were able to reach vents of hens perching at 45 cm above the floor (4 replicate pens with 14 hens each).

1.3 BONE BIOLOGY OF THE LAYING HEN

Proper skeletal development is essential to prepare the pullet to maintain high levels of egg production. To evaluate and readjust pullet feeding strategies in order to improve bone quality of the young layer, understanding the bone growth process is

necessary. Two skeletal growth phases have been identified by the increment of ash weight per day. The first phase is mainly skeletal growth due to calcification on a functional basis related to protein and fat growth. The highest deposition rate of ash during this period is from 3 to 9 wk of age. The second phase is the mineralization of the medullary bone from 16 to 21 wk of age. During this second phase, ash deposition (g/d) increased up to 19 % of total ash (Kwakkel et al., 1993).

Most bones reach their mature weight and length around 12 wk of age (Kwakkel et al., 1993). Increased bone length is a result of the division of cartilage cells in the growth plates at the end of long bones (Whitehead, 2004). Two types of bone tissue are found in the long bones of birds; cortical and trabecular. Cortical bone is the main, outer structural shell of the bone. Trabecular bone is a woven bone based on an irregular structure of collagen fibrils. As the pullet approaches sexual maturity, a series of hormonal changes results in cessation of cortical and trabecular bone growth and the growth plates become mineralized (Whitehead, 2004; Beck and Hansen, 2004).

However, at this point, bone growth is not yet complete. Approximately 10 to 14 d prior to the first oviposition (Hurwitz, 1964), pullets increase the diameter of the long bones by about 20% to deposit medullary bone (Riddell, 1992). Medullary bone is formed in female birds in response to increasing levels of estrogen associated with the onset of sexual maturity (Beck and Hansen, 2004). Medullary bone is deposited on the endosteal and trabecular surfaces, particularly of the leg bones (Whitehead, 2004). Medullary bone will continue to be deposited as long as the hen is producing high levels of estrogen (Beck and Hansen, 2004). During the laying period, cortical and trabecular bone have primarily structural roles, whereas medullary bone is a labile source of Ca to

support eggshell formation (Hurwitz, 1964).Also, during this period, some hens undergoes a loss of structural bone types (Wilson et al. 1992) resulting in osteoporosis (Whitehead, 2004). Osteporotic hens will have weaker bones and will be more likely to suffer bone fractures (Whitehead, 2004).

Bone healing process of long bone fractures consists of three distinct but overlapping stages (Kalfas, 2001): 1) the early inflammatory stage in which a hematoma develops within the fracture site during the first hours. Inflammatory cells and fibroblasts infiltrate the bone. This results in the formation of granulation tissue, ingrowth of vascular tissue, and migration of mesenchymal cells. 2) the repair stage in which fibroblasts begin to synthesizes the extracellular matrix and collagen to form a stroma, that helps support vascular ingrowth. As vascular ingrowth progresses, osteoid is secreted and subsequently mineralized, which leads to the formation of a soft callus around the repair site. Eventually, the callus ossifies, forming a bridge of woven bone between the fracture fragments. 3) the remodeling stage in which the healing bone is restored to its original shape, structure, and mechanical strength.

1.4 ASSESSMENT OF SKELETAL INTEGRITY IN LAYING HENS

Bone status can be assessed by measuring bone ash percentage, bone mineral content, bone mineral density or bone mechanical properties. Histological examination is needed for studies involving bone cell activity of specific bone surfaces and compartments (Kim et al., 2012). Incidence and severity of bone fractures could also be used as an indirect measure of overall bone quality (Donaldson et al., 2012; Toscano et al., 2013; Wilkins et al., 2004).

One of the advantages of bone ash is that it is a direct verification of the degree of mineralization of the bone tissue itself independently of all porosities. In addition, bone ash is strongly predictive of bone mineral density and bone breaking strength with correlation coefficients of 0.92 (Zhang and Coon, 1997b) and 0.77 (Hester et al., 2004), respectively. Nevertheless, it is not possible to distinguish cortical, trabecular or medullary bone compartments with bone ash assays which might be important for some studies.

To measure bone mineral content and density in laying hens, digitized fluoroscopy (Fleming et al., 2004), dual energy X-ray absorptiometry (DEXA; Hester et al., 2004) and peripheral quantitative computed tomography (pQCT; Korver et al., 2004) have been commonly used. Among all those densitometric techniques, DEXA is the standard clinical instrument used in diagnosing osteoporosis in humans. It is also used for animals such as laying hens (Hester et al., 2004). The use of photons of two different energy levels allows this technique to be used on bone surrounded by large amounts of soft tissue (Kim et al., 2012). Bone mineral density is determined by the bone mineral content relative to the two-dimensional bone area, and is expressed as g/cm^2. Some advantages of DEXA over computerized tomography are the possibility to capture whole bone images rapidly and to scan birds without anesthesia.

However, if a more detailed study about bone density of each bone compartment and cross-sectional geometry is needed, the use of pQCT might be more beneficial. Because the pQCT scan of whole bone is time consuming and increases anesthesia time if measured in vivo, consecutive slices for a region of interest (0.5 to 1.0 mm) are used

(Kim et al., 2012). Bone mineral density measured in this way is expressed as g/cm^3, a true volumetric value.

A direct method to measure strength and resistance to bone fractures is biomechanical testing. It is based on imposing loads of know magnitudes at specific rates (Turner and Burr, 1993). Long bones are typically subjected to three- or four-point bending or torsional tests; whereas vertebral bone or cylindrical bone samples are tested by compression between two parallel plates (Kim et al., 2012).

Histomorphometry provides a cheaper and more widely available technique than qQCT to evaluate bone compartment microarchitecture and volume. The most common measured variables are bone volume, trabecular number, thickness and trabecular spacing of trabecular and medullary bone (Kim et al., 2012). These two bone compartments are more sensitive to nutritional and physiological changes than cortical bone (Kim et al., 2007). Histomorphometry remains the only method to obtain bone formation rate by means of fluorochrome labels (Kim et al., 2012).

The assessment of bone damage can be performed using dissections, radiography, and palpations examinations. The keel, because of its location and high incidence of bone breakage, is the bone most commonly used for examination of bone damage in laying hens (Kappeli, et al., 2011; Toscano et al., 2013; Wilkins et al., 2004; Tarlton et al., 2013). Keel bone damage can be detected most accurately by dissection; however, this method has its limitations such as the euthanasia of several hens and the length of the process (Wilkins et al., 2004). Also, it is not possible to take repeated measures of the same hen over time.

Radiography, an imaging technique equipped with x-rays, has been used to detect fractures over time of anesthetized laying hens (Richards et al., 2011). It is a useful tool to evaluate healing process of bone fractures and the in vivo visualization of internal dorsal fractures of the keel (Richards et al., 2011); however, its practical utilization in layer houses is difficult because of equipment cost, need of anesthesia, and the length of the process.

Keel bone palpation is the most widely used method to evaluate keel bone damage for laying hens because of its low level of invasiveness, relative speed and high accessibility due to low breast muscle (Petrik et al., 2013; Tarlton et al., 2012; Wilkins et al., 2011). The accuracy (average of the apparent prevalence by the true prevalence determined by dissection) of detecting keel bone fractures of laying hens using palpation varied from 84 to 99 % depending on 8 assessor's experience (Petrik et al., 2013).

In large scale studies exanimating keel bone fractures in the field, palpation has been done at end of the lay cycle in the layer house or at the slaughter plant; therefore, the fractures were classified according to two healing stages to imply when the bone fractures occurred (Gregory et al., 1990). New fractures consisted of sharp edges that have not undergone any secondary calcification and without observable cartilaginous or bony callus formation. They presumably occurred during depopulation and transport to the slaughter plant. Old fractures consisted of undefined edges that could show cartilaginous or bony callus. They presumably occurred in the layer house weeks before depopulation and were important to the authors because they might be associated with chronic pain.

In small controlled studies, keel bone damage has been classified in fractures, deformations (S-shaped deviations) and indentations (scallop-shaped depressions) (Clark et al., 2008; Kappeli et al., 2011). Some attempts to define and quantify the severity of keel bone indentations and deviations have been done. Clark et al. (2008) measured the depth of keel bone indentation from radiographic images positioning a straight line along the ventral edge of the keel bone measuring the depth of the largest indentation. The author reported that the keel bone indentation depth ranged from 1.08 to 1.72 mm depending on the evaluated strains in cage systems. Gunnarsson et al. (2000) differentiated severity of S-shaped keel bone deformations by classifying deviation from a cranial-caudal straight line along the edge of the keel bone in low (0.5 cm), moderate (0.5-1 cm) and high (more than 1 cm).

On the other hand, other researchers used a four-point score system to evaluate in vivo keel bone status for laying hens taking into account severity of keel bone damage types. Score 1 was considered severe damage; score 2, moderate damage; score 3, slight damage; and score 4, normal keel bone (Donaldson et al., 2012; Kappeli et al., 2011; Scholz, 2007; Tarlton et al., 2012; Wahlström et al., 2001)., However, none or little explanation on the definition of these scores has been given. Wilkins et al. (2011) developed a five-point score system to evaluate keel bone breakage where score 0 indicated no fractures; score 1, a minor fracture mostly restricted to the tip of the keel; increasing severity up associated with greater callus formation with additional damage extending to the ventral edge of the keel; score 4 indicated severe o multiple fractures extended caudally associated with large deformation. Although Wilkins et al. (2011)

provided more explanation, it does not seem to account for bone deviation by itself and bone indentations or depressions.

1.5 WELFARE AND PRODUCTION ISSUES ASSOCIATED WITH BONE FRACTURES

The welfare problem of osteoporosis is generally assessed in relation to fracture incidence. Physiological similarities between birds and mammals, including humans, indicate that the nociceptors, a sensory receptor that responds to pain, present in human periosteum are also present in bird periosteum (Webster, 2004) and that bone fracture is likely to be painful in laying hens (Sandilands et al., 2009). It is possible that pain may also arise in the absence of a recent fracture from compression of the skeleton and entrapment of nerves (Whitehead, 2004).

The impact of fractures on hen welfare has been recently considered. For instance, birds with keel bone fractures take longer to reach a food reward in a runway obstacle test and their latency to leave a perch to obtain a food reward on the floor is approximately 4 times longer than those without keel bone fractures (Nasr et al., 2012a). One possible explanation was that walking and flying become mechanically impaired by an anatomical deformity of the keel bone and damage to the pectoralis major and supracoracoideus muscles that insert on it, causing subsequent muscle degeneration or disuse atrophy. However; it is also possible that ongoing pain resulting from fracture of the keel bone could contribute to decreased mobility. This, together with recent evidence that these mobility effects can be reduced by appropriated analgesic treatment (Nasr et al., 2012b), suggests that keel fractures produce pain and are an important cause of reduced bird welfare.

Bone fractures could also affect hen behavior. Fractures or deformations of leg or keel bone may interfere with hen's ability to rest on perches, and keel and wing fractures may limit the force a bird can apply to these areas during wing flapping and in attempts to generate lift for flight (Sandilands et al., 2009). Nasr et al. (2012a) indicated a highly negative relationship ($r = -0.7$) between keel bone severity and accessing a high perch (100 cm above the floor), which indicates that high resources in the hen house will be more difficult to reach for hens with keel bone fractures.

In addition, there is some evidence that hens with keel bone fractures had also poor feather condition. Habig et al. (2013) reported a low but significantly positive relationship ($r = 0.1$) between keel bone damage and breast feather loss. Also, Donaldson et al. (2012) indicated that as keel injury severity increased, overall feather coverage tended to decrease ($P = 0.056$). It was suggested that reduced mobility because of pain and discomfort increased chances of injured hens to be feather pecked by other hens. Also, it was suggested that hens with keel bone injuries could lose more feather because of stress. However, neither incidence of feather pecking or levels of stress were evaluated in these experiments.

Bone fractures are not only a welfare concern but also have an economic impact in the egg industry. Recently, a study showed that hens with keel fractures had 5 (Nasr et al., 2012) to 7% (Nasr et al., 2013) less egg production and significantly lighter body weight and consumed more feed and water than hens free from fractures. As the economic margins in egg production are very small, even small drops in egg production can have a major effect on profitability. McCoy et al. (1996) showed that 35% of mortality of caged layers was because of osteoporosis. In addition, Gregory and Wilkins

(1989) reported that in UK, 29% of caged laying hens had broken bones before slaughter, and after evisceration 98% of carcasses had broken bones.

1.6 FACTORS INFLUENCING BONE HEALTH AND CALCIUM METABOLISM

Genetics

Variability in bone mineralization (Riczu et al, 2004) and susceptibility to bone breaks (Budgell and Silversides, 2004) are influenced by genetic factors. In fact, keel bone radiography density (KRD), humeral strength (HS) and tibia bone strength (TS) of Leghorn hens have moderate heritability ranging from 0.30 to 0.45 (Bishop et al., 2000). In addition, highly-selected strains of modern laying hens have increased susceptibility to bone fractures as compared to unselected strains (Hocking et al., 2003; Budgell and Silversides, 2004). It is suggested that modern layer crossbreeds may be more susceptible to bone fragility because of selection for light weight, for energy efficiency, for early sexual maturity and for maintaining a high rate of lay over a long period of time (Sandilands et al., 2009). Bishop et al. (2000) used a bone index including three biologically meaningful and moderately heritable traits calculated by the following formula: (0.27 x KRD) + (0.37 x HS) + (0.61 x TS) – (0.25 x BW). High bone index line improved breaking strength and reduced the incidence of bone fractures (Bishop et al., 2000) without compromising egg production or egg weight (Whitehead, 2004; Fleming et al., 2006) within 5 generations in White Leghorn hens. Genetic selection may hold promise as a means to reduce the incidence of avian osteoporosis without affecting hen productivity (Whitehead, 2004).

White Leghorn and Brown crossbreed hens are the most used hens in the egg industry. They have different body weight profiles, behaviors (Silversides et al., 2012) and calcium metabolism characteristics (Franco-Jimenez et al., 2007). Thus, it is likely that bone quality is also different. Differences in body weights among strains influence bone strength. Whitehead (2004) stated that there is a positive correlation between body weight and bone strength. Brown hens presented larger total and trabecular cross-sectional areas than White hens when both were raised in individual cages (Nadeau et al., 2005). Silversides et al. (2012) also reported that Brown hens had heavier and larger total bone areas of tibias and radius as well as greater trabecular bone density of the radius than White hens. Also, humerus (Vits et al., 2005) and tibia bones of Brown hens (Habig et al., 2013) had higher bone breaking strength compared to the White hens when both were raised in furnished cages.

Tauson and Abrahamsson (1996) did not find differences in keel bone lesions between Lohmann Selected White and Isa Brown hens in conventional cages with or without perches during the layer phase. They also reported a low usage of perch at night (17 – 40 %), indicating that hens in this study might not have normal perching behavior. In contrast, recent studies showed that Brown hens had more keel bone deformities than White hens in furnished cages (Habig et al., 2013; Vits et al., 2005) and aviary systems (Wahlström et al., 2001). Differences in bone strength, the way of sitting on the perch, and body weight seem to be the main reasons for differences in keel bone deformities between the layer strains.

Alternative housing systems

Lack of exercise in conventional cages has been linked to the increased susceptibility to osteoporosis in today's commercial laying hens (Whitehead and Fleming, 2000; Jendral et al., 2008). Housing system has a significant effect on bone strength (Vits et al., 2005) and mechanical properties. Newman and Leeson (1998) and Knowles and Broom (1990) reported that bone breaking strength was found to be lower in the laying hens housed in conventional cages than in those housed in alternative systems such as aviary or floor systems. In fact, breaking strength of humerus of hens kept in battery cages was only 54% of that in layers housed with access to perches.

Fleming et al. (1994) indicated that battery caged hens had the poorest bones as assessed by measurements of trabecular bone volume, cortical thickness and three point breaking strength compared to floor or aviary housed hens. Moreover, laying hens housed in aviaries had consistently higher bone strength compared to hens in conventional and furnished cages (Leyendecker et al., 2005). The tibia and humerus of two-year-old laying hens raised in a free-range system had superior mechanical properties, larger cortical regions, and higher trabecular thickness and trabecular bone volume compared to hens raised in conventional cages (Shipov et al., 2010). In a meta-analysis of ten laying hen flocks from 1999 to 2006, aviary and free-range hens had the highest humerus strength. Also, aviary hens had higher tibia strength than those housed in conventional cages and in some furnished cages (Scholz, 2007).

Better bone strength in alternative housing systems is attributed to more space as well as different equipment such as perches, sand baths and nests (Abrahamsson et al., 1996). There is a positive relationship between high frequency of perch usage and

increased bone strength (Hughes and Appleby, 1989) and increased trabecular bone volume (Wilson et al., 1992). The provision of perches has been demonstrated to slow down the loss of structural bone compared with birds housed in conventional cages although it does not prevent bones from becoming osteoporotic (Wilson et al., 1993). However, perch provision alone is not enough. The amount of movement birds can perform also seems to be important, as birds housed in cages with perches tend to have weaker bones than those housed in extensive systems with perches (Leyendecker et al., 2005).

Although bones of hens in alternative housing are stronger than those of caged hens, hens in alternative housing systems have still a higher incidence of old fractures (Fleming et al., 2006; Wilkins et al., 2004). The proportion of birds with old breaks was 73 % in a perchery system (Freire et al., 2003); between 49 and 67 % in commercial single-tier, wire floor system (Nicol et al., 2006); between 50 and 78 % in free-range systems (Wilkins et al., 2004); and 62 % in furnished cages (Sherwin et al., 2010).

The majority of breaks sustained by laying hens are to the furcula and the keel in free range and aviary systems (Wilkins et al., 2004). From a full dissection of 50 free-range hens with bone fractures, 6.9 % of hens had keel bone fractures, 4.5 % had furcula fractures; the rest of bone fractures were present only in 0.8 % of hens with bone fractures (Gregory et al., 1990). The incidence of old keel breaks of hens in non-cage systems ranges from 52 to 86% (Freire et al., 2003; Nicol et al., 2006; Wilkins et al., 2011). Incidence of keel bone deformities and fractures is low during the pullet rearing phase, but it increases during the layer phase. Enneking et al. (2012) reported that bone fracture did not occur in caged White pullet flocks prior to 12 wk of age regardless of

whether pullets had access to a perch or not. Similarly, pullets of egg laying strains reared in a variety of housing systems showed a very low incidence (0.8 %) of keel bone deformities at 15 wk of age (Fleming et al., 2004).

On the other hand, Kappeli et al. (2011) reported that White pullets raised in aviary systems showed a low incidence (7 %) of slightly deformed keel bones at 12 wk and a very low incidence (2%) at 18 wk of age, and no keel bone fractures were observed during the pullet rearing phase. Studies conducted during the layer phase indicated that fractures of ISA Brown hens housed with access to perches occurred between 25 and 45 weeks of age(2 %), and almost doubled from 59 to 72 wk of age (14 to 23 %) (Gregory and Wilkins, 1996). In agreement with previous experiments, Scholz (2007) and Kappeli et al. (2011) reported that keel bone deformities significantly increase during the laying period.

Even though perching seems to have a positive influence on bone strength, it is also commonly associated with higher incidence of keel bone deformations. Pickel et al. (2011) found that peak force on the keel bone in hens sitting on different types of perches was approximately 5 times higher than peak force on a single foot pad. The high and long-term pressure load on the keel bone during perching may be responsible for keel bone deformities in laying hens. Sandilands et al. (2010) reported that 39% of high (60 cm) jumps resulted in poor landing versus just 2% of low (30 cm) jumps; however, keel bone damage was similar between hens with (36 %) or without (32 %) perches, suggesting that perches per se are not necessarily responsible for keel damage. Hens bumping into other equipment such nests, slats or feeders might be partially responsible for the high incidence of keel bone injuries in alternative housing systems.

The effects of exercise as a mean of stimulating bone growth during rearing have been studied. Vits et al. (2005) reported that both humerus and tibia strength in hens housed in furnished cages was higher when pullets were reared in cages than on the floor. In another study, humerus strength was also higher in caged hens reared in pullet cages than in those reared on the deep litter floor (Gregory et al., 1991). At the beginning of the laying period, Vits et al. (2005) observed that hens reared on the floor were unwilling to move on the wire floor of the furnished cages Therefore, it is possible that higher activity and faster adaptation of caged pullets to furnished or conventional cages at the beginning of the laying period compared to the floor-raised pullets might have influenced bone strength during the layer phase. Evaluating the effects of pullet housing systems on bone strength during the layer stage could have some confounding effects such as adaptability of hens to the new layer environment. The access of perch in cages during the pullet rearing phase increased bone mineral content of tibias, keel bone, and humerus at 12 wk of age (Enneking et al., 2012) and increased keel bone mineral density at 50 wk of age; however, it elevated the incidence of keel bone fractures and deviations of 71-wk-old hens, regardless of perch access during the layer phase (Hester et al., 2013).

Although improved bone quality in alternative housing systems has been primarily associated to increased exercise, recycling P or Ca from litter in floor pens might increase intake of both minerals and possibly improve skeletal integrity in housing systems with litter access. For instance, in concrete pens with raised wire floor with access to old litter (1.86 % Ca and 0.49 % P) in a feeder, hens fed the two lower Ca levels (1.00 and 0.25 %) had higher litter consumption compared to hens fed the highest Ca level (3.22 %) (Harms et al., 1984). In addition, hens housed in concrete pens with raised

wire floor with access to litter in a feeder had higher egg specific gravity when fed any of the dietary calcium levels compared to those housed without access to litter in a feeder (Harms et al., 1984).

Housing systems might also affect skeletal integrity because of changes in calcium retention. Calcium retention calculated using the amount of calcium consumed and subtracting the amount of calcium that was deposited in the egg and excreted in the manure was compared between conventional and enriched cages (Neijat et al., 2011). Hens housed in enriched cages had overall higher calcium retention because of reduced manure Ca excretion and similar Ca outputs in eggs (Neijat et al., 2011); however, the biological mechanism by which environment enrichment may affect calcium retention was not provided.

Particle size of calcium carbonate during pullet rearing phase

Using isotope tracer (Ca^{45}) technique, it has been shown that during the early production period the young hen is in a negative calcium balance suggesting that calcium was mobilized from bone reserves for eggshell formation (Mueller et al., 1964). The author also indicated that 36 % of calcium used for secretion of calcium into the eggshell came from the skeletal calcium reserves and the rest came from the feed. Thus, calcium body reserves during the pullet rearing phase could be important to sustain bone integrity during the layer phase.

Limestone is the most common Ca source used in poultry diets. Particle size of limestone influences gastrointestinal passage and the rate of Ca dissociation in the gizzard and proventriculus. Zhang and Coon (1997a) reported that a limestone particle size greater than 0.8 mm accumulates in the gizzard for a prolonged period of time. This

retention allowed Ca to dissolve slowly and enter into the intestine at a slower rate. The gastric acidity of hydrochloric acid in the proventriculus and gizzard converts the cations temporarily into chloride salts, that have a considerable degree of ionic dissociation (Leeson and Summers, 2001). As Ca is absorbed in its ionic form in the jejunum, limestone particle size can affect calcium absorption rate.

For 28-d-old broilers, Guinotte et al. (1991) showed that a fine (< 0.15 mm) limestone rather than a medium (0.6 to 1.18 mm) sized or coarse (> 1.18 mm) particle size increased tibia length and stiffness. On the other hand, McNaughton et al. (1981) evaluated a fine (0.07 - 0.15 mm), a medium (0.25 - 0.84 mm) and a coarse (0.84 - 1.7 mm) limestone particle size from 1 to 21 d of age for broilers. The author indicated that tibia ash % was the highest when broilers were fed a limestone particle size ranged from 0.25 to 0.84 mm. Coon and Manangi (2006) showed that the use of a 0.38 mm limestone particle size tended to have higher tibia ash/tibia mg compared to the use of 0.03 mm, 0.80 or 1.31 mm limestone particle size of 28-day-old broiler chickens fed diets with phytase. Discrepancies of limestone particle size studies for broilers might come from differences in ages, particle size ranges and phytase use.

Phytase is an enzyme regularly used in poultry diets nowadays. There are several studies indicating that calcium level affect efficacy of phytase (Selle and Ravindran, 2007). High calcium level has been associated with lower phytase efficiency because calcium and phytate can form insoluble complexes reducing phytase substrate (Tamim et al., 2004). Thus, a larger or medium rather than fine particle size of limestone will allow a slowly release of Ca into the intestine preventing the formation of insoluble Ca-phytate phosphorus complexes that are not absorbed in the intestine (Selle and Ravindran, 2007).

There are limited studies of limestone particle size for pullets. Finding in broiler studies of limestone particle size might not extrapolate to pullets because they have different growth rate. Geraldo et al. (2004) studied the interaction between two particle size of limestone and five levels of dietary calcium from 3 to 16 wk of age in cage systems. A medium sized particle of limestone (0.899 mm), but not a fine particle size (0.135 mm), increased tibia ash at 16 wk of age and at calcium content of tibia at 30 wk of age when both bird groups were fed 0.90 % of dietary Ca. However, there is no study of limestone particle size during the pullet phase in alternative housing systems.

1.7 SUMMARY

Because of consumer preference, increased hen welfare legislation, and industry awareness of animal well-being, alternative housing systems, particularly enriched colony cages, are becoming more widespread in the near future in the U.S. Even though the use of alternative housing systems has positive effects on some aspects of hen welfare, they had a high incidence of bone fractures.

Bone quality is influenced by genetics, housing system environment and nutrition. Increased floor space allowance and usage of resources, such as perches, nest boxes and dust bathing area, in alternative housing systems have been associated with improvements in bone strength. However, higher incidence of old bone fractures has been reported in alternative housing systems because of the increased complexity of the environment. Particle size of limestone during the pullet rearing phase influenced bone integrity in caged hens. However, no experiments have examined the effect of this nutritional strategy in deep litter or aviary systems in comparison with conventional

cages. There are still management and nutritional concerns as producers move away from conventional cages to alternative housing systems.

1.8 RESEARCH OBJECTIVES

Some authors have evaluated the effects of cage-free housing systems in comparison with conventional cages on performance and bone health resulting in conflicting results. Also, most of them were conducted in Europe and used predominantly Brown hens. Furthermore, there is little research on specific keel bone injury, such as twists, indentations or fractures, in alternative systems. Therefore, our first objective was to investigate the effects of cage-free housing systems on hen performance and bone health of White hens under US conditions (Chapter 2).

As pullets undergo fast bone formation during the pullet rearing phase, nutritional strategies during this phase could have a major impact on bone quality. Recent studies indicate that a medium, but not a fine, particle size of limestone during the pullet phase is beneficial to bone health of pullets and hens in conventional cages, but there is no investigation of this nutritional strategy in alternative housing systems. Thus, our second objective was to evaluate the effect of particle size of limestone provided from 7 to 17 wk of age to White and Brown pullets in conventional cage and aviary systems during the pullet (Chapter 3) and layer (Chapter 4) phase. Our third objective was to evaluate the effect of particle size of limestone provided from 9 to 17 wk of age in deep litter systems to White pullets during the pullet and layer phase (Chapter 5).

Emphasis in both nutritional studies was given to bone health measured by bone mineral density and incidence of keel bone deformities and fractures. Eggshell quality

was also evaluated as bone mineralization also affects calcium metabolism and availability for eggshell formation. Usage of resources (nest boxes and perches) was also analyzed, as changes in activity between White and Brown strains in alternative housing systems could also alter bone integrity (Chapter 6).

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CHAPTER 2. EVALUATION OF HEN PRODUCTIVITY, EGGSHELL QUALITY, AND BONE HEALTH OF WHITE LAYING HENS HOUSED IN CONVENTIONAL CAGES OR LITTER FLOOR PENS

2.1. INTRODUCTION

Conventional cage systems have been the most popular housing systems for laying hens in the United States for more than fifty years. However, in the last few years, increased animal welfare concerns and the ban of battery cages in some regions are leading egg producers to switch to alternative housing systems. One of those alternative housing systems is the deep litter system. This system is becoming more widespread and despite the low stocking densities compared to other alternative housing systems it could be an economically viable investment because of its low cost to build.

In cage free housing systems, hens are provided with more space and have access to a nest, a perch and a litter area for pecking, scratching and dust bathing. Several authors have demonstrated that non-cage housing systems increase comfort behaviors of hens compared to conventional cages (Tanaka and Hurnik, 1992; Shimmura et al., 2010, Freire and Cowling, 2013). However, there is still no consensus about the effects of housing systems on hen productivity and mortality. Some research has indicated that hen performance and livability could be detrimentally affected in barn or deep litter systems in contrast to conventional caged systems (Sherwin et al., 2010; Weeks, 2012; Stanley, 2013, Freire and Cowling, 2013) while others found no difference in hen performance between cages and litter floor pens (Aerni et al., 2005, Singh et al., 2009; Scholz, 2008).

One of the major welfare concerns associated with conventional cage systems has been bone fractures due to osteoporosis during the later stages of egg production (Whitehead, 2004). While bone strength is improved in alternative housing systems

(Freire and Cowling, 2013) due to increased activity, prevalence of bone fractures is still high and proposes a serious welfare problem (Fleming et al., 2006; Sandilands, 2009). Part of the skeleton that suffers the most fractures is the keel bone accounting for 90 % of old fractures in cage free systems (Wilkins et al., 2004). Nicol et al. (2006) reported that the incidence of keel breaks range from 52 to 73 % in non-cage housing systems during the laying period.

Osteoporosis and poor bone density can translate to poor eggshell quality. Compromised eggshell strength is highly linked to increased percentage of cracked eggs that results in financial losses. Some studies have demonstrated higher eggshell strength and eggshell percentage in eggs from cage hens compared to hens housed in litter floor pens (Pistěková et al., 2006, Tůmová et al., 2011). On the other hand, other experiments have not shown differences in eggshell strength due to housing systems (Petek et al., 2009; Dukić-Stojčić et al., 2009). Because of conflicting research results of comparative housing systems on eggshell quality, there is a need to further investigate the effects of cage free systems on bone quality and related eggshell strength.

Most of the comparative studies of housing systems have been done in Europe which has distinctive management practices such as the usage of wheat rather than corn as main grain source and the predominant utilization of Brown Leghorn rather than White Leghorn hens. The objective of this pilot study was to evaluate hen productivity, bone health, and egg quality of White Leghorn laying hens housed in conventional caged or deep litter system from 32 to 47 wk of age under US conditions.

2.2. MATERIALS AND METHODS

Birds and Husbandry

At 32 wk of age, Bovan White Leghorn beak-trimmed laying hens were moved from a commercial style cage system to new cages in a three tier battery cage system or wood-shaving-litter floor pens located in the same tunnel-ventilated room. Hens were given one week for acclimatization to the new facility. The experiment was conducted mid-spring to late-summer. Hens were maintained on a 16L:8D photoperiod. A total of 232 hens were equally distributed into 8 litter floor pens (29 hens/floor pen). Each floor pen (1608 cm^2/hen) was furnished with six plastic nest boxes (Chick Box™, Broiler Equipment Company, Shropshire, England) arranged in two levels (15 cm and 50 cm; nest height measured from the litter floor to the plastic perch attached to each nest box) providing 192 cm^2/hen of nest area, one 15-kg-tube feeder (4.8 cm/hen), four nipple drinkers, and two-tier (43 cm and 80 cm above the floor) rectangular wooden perches. These perches along with perches attached to nest boxes provided 15 cm of perch space per hen. Additionally, 32 caged hens were equally distributed into 8 conventional cages (463 cm^2/hen). Each cage was equipped with one nipple drinker, one feeder trough (5 cm/hen), and a manure tray. Hens were weighed individually at 33, 38, 43 and 47 wk of age to calculate body weight gain (BWG). Feed intake was calculated by feed disappearance weekly. Calcium intake was calculated by multiplying feed intake by calcium content in the diet.

At 37 wk of age, number of hens roosting at high (80 cm), intermediate (43 and 50 cm) and lower perches (15 cm) was recorded at 11:00 PM, two hours after lights were off. Number of eggs and the location laid were recorded daily. A sample of 12 eggs from

each floor pen and 4 eggs from each cage were collected to weigh at 38, 43, and 47 wk of age. Egg mass was calculated by multiplying average egg weight by hen-day egg production. Feed conversion ratio was calculated by dividing average daily feed intake and average daily egg mass.

Hens were fed a corn-soybean meal based diet in mash form to meet Bovan nutrient recommendations and NRC poultry nutrition recommendations (Table 2.1). Because of an early outbreak of coccidiosis during our study, Amprolium was included at 0.0125 % level from 39 wk of age until the end of the trial. All procedures were approved by the University of Nebraska-Lincoln Institute of Animal Care and Use Committee.

Eggshell characteristics

At 37 and 43 wk of age, 12 eggs from each floor pen and 4 eggs from each cage were collected to measure eggshell percentage. Immediately after egg collection, eggs were broken open, albumen and yolk were removed, and eggshells were weighed. Eggshell percentage was expressed as a percentage of initial egg weight. Eggshell mass was calculated by multiplying eggshell weight by hen-day egg production of the respective week. Estimated calcium needed for eggshell formation was calculated by multiplying eggshell mass by reference eggshell calcium content (97%) (Burley and Vadehra, 1989). At 37, 41, and 46 wk of age, 12 eggs from each floor pen and 4 eggs from each cage were sampled to evaluate eggshell breaking strength using a Texture Analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY) measuring force (Newton, Kg . m/s^2) to break the eggshell.

Bone health examination

At 38, 42, and 46 wk of age, all hens from the study were palpated to examine keel bone status. The palpation involved running 2 fingers down the side of the keel bone and feeling for the present of twists (curved keel bones), indentations (depressions with undefined edges) or fractures (sharp edges without palpable callus formation) (Clark et al., 2008).

At 48 wk of age, a sample of two randomly selected hens per replicate were scanned using a dual-emission x-ray absorptiometer (Model No. 476D014, Norland Medical Systems, Fort Atkinson, WI) to determine in-vivo bone mineral density (BMD), bone mineral content (BMC) and bone area of right tibias including fibulas. Unanesthetized laying hens were placed facing up on a foam device and restrained with Velcro straps around the neck, breast including the wings and shanks for 12 minutes while the scan was taken (Hester et al., 2004).

Statistical Analysis

A completely randomized design with repeated measures was used for performance, egg quality, bone characteristics and prevalence of keel bone deformities. Repeated measures analysis was conducted to determine changes in data throughout the course of the study. As repeated measures from the same subject are usually dependent, the measurements from the same subject over time might be correlated. To evaluate this correlation structure for each variable the following covariance patterns were tested: 1) compound symmetry, 2) autoregressive of order 1, 3) toeplitz, and 4) unstructured, using the AICC (AIC, Akaike information criterion, with a correction for finite sample sizes) to

select the best fit for the model. BW was used as a covariate to evaluate bone mineral density, content and area.

Prevalence of each keel bone deformity or injury was analyzed using a binomial logistic regression analysis because this variable was a designation of one of two possible outcomes (binary response), pullets having a specific keel bone issue or pullets having a normal keel bone without any keel bone damage. This analysis resulted in the generation of odds and odds ratio (Szumilas, 2010). Odds (o) are the probability (p) of having a specific keel bone issue over not having it ($1 - p$). Probability of having a specific keel bone issue can be calculated using odds following this formula: $p = o / (1 + o)$. While probabilities range from 0 to 1, odds range from 0 to positive infinity. Odds ratio served as a comparative measure of odds between two treatment groups.

Nest and perch preference was analyzed using a Poisson regression analysis for count data. Poisson distribution was implemented to evaluate the rate of occurrence of an event estimated by relating the logarithmic transformation of predicted value to a linear function (Petrie and Watson, 2013). The relative rate represents the ratio of rates between two treatment groups (Petrie and Watson, 2013). Means were separated using LS means statement. Differences between means were considered statistically significant if the $P \leq 0.10$.

2.3. RESULTS

Performance

There was an interaction ($P < 0.0001$) between housing system and age on BW (Figure 2.1). At the start of the trial (33 wk of age), caged hens had lower (1505 vs. 1561

g; $P = 0.058$) BW than floor housed hens but then gained more BW than floor housed hens during the trial (Table 2.2, Figure 2.1). This was primarily due to differences in BWG during the first five weeks of this trial ($P < 0.0001$) (Table 2.2). From 33 to 37 wk of age, floor housed hens lost 8.31 g of BW whereas caged hens gained 134.94 g. From 38 wk to the end of the trial, BWG remained similar between the two groups of hens.

Mortality occurred in only two of the eight floor pens. It is worth noting that none of the caged hens died during the trial whereas 11 out of 232 floor-housed hens (4.74 %) died from 36 to 40 wk of age (Figure 2.2). All dead laying hens showed sign of diarrhea. The presence of orange or red tint in feces along with the diagnosis of coccidiosis from two hens confirmed the outbreak of coccidiosis. Figure 2.2 shows mortality rate over time in floor pens.

Feed intake and calcium feed intake increased from 33 to 47 wk of age ($P < 0.0001$) (Table 2.2). However, housing system did not affect feed intake and calcium feed intake at any age ($P = 0.244$). An interaction ($P = 0.005$) between housing system and age indicated that caged hens had lower FCR than floor housed hens throughout the trial. Also, FCR of caged hens did not change over time but FCR of floor housed hens reduced during the last period of this trial (43 to 47 wk).

An interaction ($P < 0.0001$) between housing system and age indicated that even though caged hens had higher hen-day egg production than floor housed hens throughout the trial, hen-day egg production of floor housed hens improved during the last period of this trial (43 to 47 wk). In addition, an interaction ($P < 0.0001$) between housing system and age indicated that floor housed hens produced less egg mass compared to caged hens

throughout the trial and egg mass of both caged and floor housed hens increased during the last 5 wk period.

Eggshell measurements

An interaction ($P = 0.063$) between housing system and age for egg weight indicated that weights of eggs from caged hens increased from 37 to 43 wk of age whereas those from floor housed hens remained the same (Table 2.3). Housing system influenced eggshell weight ($P = 0.073$), eggshell percentage ($P = 0.048$), eggshell mass ($P < 0.0001$), and estimated calcium for eggshell formation ($P < 0.0001$). Caged hens produced eggs with higher eggshell weight, percentage and mass as well as higher estimated calcium for eggshell formation compared to eggs from floor housed hens. Percentage of eggshell reduced ($P = 0.006$) from 37 to 43 wk of age. An interaction between housing system and age ($P = 0.020$) indicated that caged hens produced eggs with stronger eggshells than those from floor housed hens at 41 wk of age ($P = 0.015$); however, this difference in eggshell breaking strength was not observed at 37 and 46 wk of age (Figure 2.3).

Preference of nests and perches in floor pens

Only 12 percent of hens in floor pens were perching at 37 wk of age during the night. This low usage of perches was observed by workers until the end of the trial. As none of hens used the intermediate wooden perch (43 cm) at 37 wk of age, comparison analysis of height preference of perches was conducted among high (80 cm), intermediate (50 cm) and low perches (15 cm) (Table 4). There was an effect of perch height on rate of perching ($P = 0.026$). The rate of perching in intermediate perches was 4.25 times the rate of perching in high perches ($P = 0.017$) whereas the rate of perching in low or high

perches was similar ($P = 0.382$). The rate of perching in low perches was less than half the rate of perching in intermediate perches ($P = 0.062$).

Only 10 percent of hens were using nest boxes at the start of the trial and this percentage increased up to 15 percent at the end of the trial. There was an interaction ($P = 0.036$) between nest height and age on rate of laying (Figure 2.4). At any age, the rate of laying in lower nest boxes were higher than the rate of laying in upper nest boxes. However, the acceptance of higher nest boxes increased over time while the usage of lower nest boxes increased only the first two weeks of the study and then remained similar throughout the trial (Figure 2.4).

Bone health

There was an effect ($P = 0.104$) of housing system on odds of observing keel bone indentations indicating that floor housed hens had greater chances of having keel bone indentations than caged hens (0.06 vs. 0.13) (Table 2.5). The chance to observe hens with keel bone deformities in deep litter systems were 2.10 times higher than in conventional cage systems (Table 2.5). Housing system did not influence the odds of observing fractured ($P = 0.334$) or curved ($P = 0.305$) keel bones. Higher odds of observing fractured keel bones were observed at 38 wk of age compared to at 43 wk of age and intermediate values were observed at 47 wk of age ($P = 0.077$). At 47 wk of age, floor housed hens had greater bone areas (4.56 vs. 3.31; $P = 0.065$) and higher BMC (1.10 vs. 0.75; $P = 0.040$) than caged hens. However, housing system did not affect BMD ($P = 0.485$) (Table 2.6).

2.4. DISCUSSION

Performance

During the first 5-wk period of this study, floor housed hens lost 8.31 g of BW and caged hens gained 135 g of BW resulting in differences in BW in favor of caged hens that lasted throughout this study. However, feed intake was not affected by housing system as previously reported by Singh et al. (2009) and Golden et al. (2012). Because hens were fed ad libitum and they did not differ in feed consumption, changes in initial BGW might have been caused by depression of immune function due to stress along with increased pathogen contamination. In fact, there was numerically higher hen mortality in floor pens (4.74 %) compared to conventional cages (0.00 %) due to diagnosed coccidiosis suggesting that floor housed hens had potentially higher risk of contracting coccidiosis. Coccidiosis is a parasitic disease that causes intestine mucosal damage affecting negatively absorption of nutrients (Yegani and Korver, 2008).

Caged hens had higher hen-day egg production, egg mass, and improved feed conversion ratio compared to than floor housed hens from 33 to 47 wk of age. Similarly, higher egg production and egg mass were observed in conventional cages than in deep litter systems by other researchers (Yakubu et al., 2007; Voslářová et al., 2006; Stanley et al., 2013). In fact, a quantitative comparison of 35 comparative studies of housing systems on hen performance from 1974 to 2011 showed that caged hens had higher egg production than floor housed hens (Freire and Cowling, 2013). None of those authors reported effects of housing systems on feed efficiency. On the other hand, Singh et al. (2009) and Shimmura et al. (2010) did not find differences in feed efficiency between caged and deep litter systems. However, it is important to mention that floor space

allowance in floor pens was about three to five times higher in cited papers compared to this study.

After the first 5 wk of this trial, body weight gains of hens were similar between both housing systems indicating that hens were adjusting to their new environments. Regardless of housing system, feed intake increased over time during this trial. Even though hen-day egg production and feed conversion ratio deteriorated in litter floor pens, there were improvements of these variables only in this housing system during the last 5-wk period of this study. In line with these observations, a longer experiment conducted from 20 to 50 wk of age by Singh et al. (2009) indicated that cage hens had higher initial egg production, floor housed hens caught up, and then exceeded cage hens at the final time point resulting in no differences in overall egg production for entire trial.

Eggshell characteristics

Our results were in agreement with Van den brand et al. (2004) and Petek et al. (2009) work. The authors found no significant difference in egg weight produced by caged hens versus free range hens. Although egg weights were similar between housing systems at 37 and 46 wk of age, floor housed eggs had lower overall eggshell percentage and decreased eggshell strength at only 41 wk of age. In contrast to our results, some researchers demonstrated that floor housed hens had higher eggshell percentage than caged hens (Hu, 2013; Golden et al., 2012, Stanley; 2013). Golden et al. (2012) suggested that floor housed hens could obtain more calcium from soil through coprophagia. In this experiment, coccidiosis infection during the middle of the trial might have affected overall nutrient digestibility.

Nest and perch preference in floor pens

The low usage of nest and perches in floor pens might be associated with the late exposure to this equipment after the start of lay. The low usage of high perches and nest boxes could indicate impairment of spatial skills of laying hens as previously reported by Gunnarsson et al. (2000). However, as the hens aged, the usage of higher nest boxes increased indicating the floor hens were gradually developing spatial skills.

Bone health

Housing system only affected the odds of observing hens with keel bone indentation, being greater in floor pens compared to conventional cages. Shimmura et al. (2010) did not find significant differences of keel bone deformities score between conventional cages and single tier aviary system. But, it is important to mention that they did not evaluate specific prevalence of keel bone deformities or fractures that could give further information regarding keel bone integrity. Several other researchers have reported higher incidence of keel bone deformities or fractures in alternative housing systems than in conventional cages (Wilkins et al., 2004; Nicol et al., 2006; Sherwin et al., 2010).

After 14 wk of movement from cages to floor pens, hens had higher bone area and mineral content but bone mineral density was not affected. Newman and Leeson (1998) reported that tibia strength increased within only 20 d of transferring 69-wk old hens from cages to an aviary. Wilson et al. (1992) demonstrated that there is a high positive relationship between high frequency of perch usage and increased trabecular bone volume. Thus, it seems that the increased activity due to greater floor space and roosting influenced positively tibia bone integrity of hens housed in floor pens. This finding

suggests that this mechanism may involve some stimulation of structural bone formation but bone mineralization rate might not have been affected.

2.5. CONCLUSIONS

Major effects of housing systems were observed on hen performance during the first 5 wk of this study. Hens of both housing systems seemed to adjust to some degree to their environments in terms of hen productivity. The use of a deep litter system negatively affected eggshell percentage and eggshell strength likely due to the coccidiosis challenge observed in this system.

These findings suggest that housing system affected structural bone remodeling to some degree; however, it is possible that the increase in bone area and mineral content could have been associated with temporary decreased of egg production in caged hens transferred to litter floor pens, a radically different environment. The results of this experiment strictly apply only to Bovan White laying hens and similar investigations should be performed in other strains of laying hens. Further investigation is necessary to improve design and management practices in floor pens to prevent economic losses.

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Table 2.1. Diet composition and calculated nutrient content (as-fed basis)

Ingredients (%)	Quantity
Corn	53.37
Soybean meal	18.37
DDGS	15.00
Corn oil	2.07
Dicalcium phosphate	0.68
Fine limestone[1]	3.89
Shell and bone builder limestone[1]	5.83
Salt	0.34
Lysine	0.15
Methionine	0.10
Vitamin & Mineral premix[2]	0.20
Calculated nutrients	
Metabolizable energy, kcal/kg	2,775
Crude protein, %	17.00
Methionine, %	0.39
Methionine+Cysteine, %	0.74
Lysine, %	0.90
Tryptophan, %	0.19
Threonine, %	0.66
Ca, %	4.01
Available P, %	0.40
Sodium, %	0.18

Ronozyme (500 FTU/g) was considered to release 0.1 % of Ca and P.
Amprolium was included at 0.0125 % level from 39 wk of age until the end of the trial.
[1] ILC Resources, Weeping Water, NE.
[2]Vitamin and trace minerals provided the following per kilogram of feed: Vitamin A (retinyl acetate, 6,600 IU); vitamin D_3 (cholecalciferol, 2,805 IU); vitamin E (DL-α-tocopheryl acetate, 10 IU); vitamin K_3 (menadione dimethpyrimidinol, 2.0 mg); vitamin B_2 (riboflavin, 4.4 mg); vitamin B_5 (pantothenic acid 6.6 mg); Vitamin B_3 (niacin, 24.2 mg); vitamin B_7 (biotin, 26 mg); vitamin B_{12} (cobalamin, 6 mg); and choline ($C_5H_{14}ClNO$, 322 mg). Mn (MnO, 54 mg); Cu ($CuSO_4H_2O$, 6.6 mg); Fe ($FeSO_4H2O$, 22 mg); Zn (ZnO, 50 mg); and Se (Na_2SeO_3, 0.24 mg).

Table 2.2. Effects of housing system on body weight gain, feed intake, feed conversion ratio, hen day egg production and egg mass.

Housing system	Age (wk)	Body weight gain (g)	Feed intake (g/bird/day)	Calcium intake[1] (g/bird/day)	Feed conversion ratio (g/g)	Hen day egg production (%)	Egg mass (g)
Cage		84.18^{a}	114.14	4.58	1.892^{b}	96.88^{a}	61.17^{a}
Floor		38.89^{b}	111.72	4.48	2.183^{a}	81.18^{b}	51.47^{b}
		(6.64)	(1.42)	(0.06)	(0.032)	(1.44)	(0.96)
	33-37	63.31	107.69^{c}	4.32^{c}	2.038^{ab}	87.87^{b}	53.98^{b}
	38-42	46.02	112.10^{b}	4.50^{b}	2.076^{a}	87.74^{b}	55.25^{b}
	43-47	75.24	119.00^{a}	4.77^{a}	2.000^{b}	91.48^{a}	59.74^{a}
		(10.81)	(1.34)	(0.05)	(0.029)	(1.16)	(0.83)
Cage	33-37	134.94^{a}	110.06	4.41	1.885^{c}	96.33^{a}	59.67^{bc}
	38-42	35.59^{bc}	113.48	4.55	1.883^{c}	98.19^{a}	61.39^{ab}
	43-47	82.00^{b}	118.89	4.77	1.909^{c}	96.13^{a}	62.47^{a}
Floor	33-37	-8.31^{c}	105.33	4.22	2.190^{a}	79.41^{c}	48.29^{d}
	38-42	56.45^{b}	110.72	4.44	2.269^{a}	77.28^{c}	49.10^{d}
	43-47	68.49^{b}	119.11	4.77	2.091^{b}	86.84^{b}	57.02^{c}
		(15.29)	(1.89)	(0.08)	(0.041)	(1.84)	(1.17)
Source of variation					*P*-values		
Housing system		0.0002	0.244	0.244	< 0.0001	< 0.0001	< 0.0001
Age		0.293	<0.0001	< 0.0001	0.041	0.0002	< 0.0001
Housing system × age		0.0001	0.433	0.434	0.005	< 0.0001	< 0.0001

[1] Calcium intake was calculated by multiplying feed intake by Ca content in the diet. $^{a - c}$ Means with different superscripts in the same column differ at $P \leq 0.05$. $^{A, B}$ Means with different superscripts in the same column differ at $P \leq 0.10$. Values in parentheses are SEM.

Table 2.3. Effect of housing system on egg weight, eggshell weight, eggshell percentage, eggshell mass, and estimated calcium for eggshell formation

Housing system	Age (wk)	Egg weight (g)	Eggshell (%)	Eggshell weight (g)	Eggshell mass[1] (g)	Estimated calcium for eggshell formation[2] (g)
Cage		60.70	11.40[a]	6.92[a]	6.31[a]	6.12[a]
Floor		60.70	11.15[b]	6.76[b]	5.50[b]	5.33[b]
		(0.47)	(0.09)	(0.06)	(0.13)	(0.12)
	1	59.99[b]	11.43[a]	6.85	5.80[b]	5.63
	2	61.41[a]	11.12[b]	6.82	6.01[a]	5.83
		(0.47)	(0.09)	(0.06)	(0.13)	(0.12)
Cage	1	59.34[b]	11.55	6.87	6.17	5.99
	2	62.05[a]	11.25	6.96	6.45	6.26
Floor	1	60.65[ab]	11.30	6.84	5.43	5.26
	2	60.76[ab]	11.00	6.68	5.57	5.40
		(0.67)	(0.12)	(0.08)	(0.18)	(0.17)
Source of variation				*P*-values		
Housing system		0.989	0.048	0.073	< 0.0001	< 0.0001
Age		0.043	0.017	0.712	0.247	0.247
Housing System × age		0.063	0.988	0.140	0.714	0.713

[1] Eggshell mass was calculated by multiplying eggshell weight by hen day egg production. [2] Estimated calcium for eggshell formation was calculated by multiplying eggshell mass by reference eggshell calcium content (97%) (Burley and Vadehra , 1989). [a, b] Means with different superscripts in the same column differ at $P \leq 0.05$. [A, B] Means with different superscripts in the same column differ at $P \leq 0.10$. Values in parentheses are SEM.

Table 2.4. Predicted number of hens perching in each perch location in floor pens at 37 wk of age

Perch location	Mean	CI[5]
	Predicted number of hens perching	
Upper[1]	0.50[b]	0.18-1.41
Intermediate[2]	2.13[a]	1.28-3.52
Lower[3]	0.88[ab]	0.40-1.92
	Relative rate[4]	
Lower vs. Intermediate	0.41	0.16-1.05
Lower vs. Upper	1.75	0.48-6.44
Intermediate vs. Upper	4.25	1.34-13.50
Source of variation	*P*-value	
Perch location	0.026	

[1] Rectangular wooden perch at 80 cm above the litter floor.
[2] Plastic perch attached to nest boxes at 50 cm above the litter floor.
[3] Plastic perch attached to nest boxes at 15 cm above the litter floor.
[4] Ratio between the rates of two treatment groups.
[5] Confidence interval
[a,b] Means with different superscripts in the same column differ at $P \leq 0.05$.

Table 2.5. Effect of housing system on incidence of keel bone indentations, and curved keel bones or fractures

Age (wk)	Housing system	Keel bone indentations		Curved keel bones		Keel bone fractures	
		Mean	CI[3]	Mean	CI[3]	Mean	CI[3]
				Odds[1]			
	Cage	0.06[B]	0.02-0.15	0.22	0.12-0.41	0.07	0.03-0.16
	Floor	0.13[A]	0.10-0.16	0.16	0.12-0.21	0.10	0.08-0.13
38		0.06	0.02-0.16	0.15	0.08-0.28	0.15[a]	0.09-0.26
42		0.09	0.05-0.19	0.19	0.11-0.32	0.05[b]	0.02-0.12
46		0.12	0.07-0.21	0.23	0.14-0.38	0.08[ab]	0.04-0.16
				Odds ratio[2]			
	Floor vs. Cage	2.10	0.84-5.27	0.72	0.36-1.40	1.53	0.62-3.80
38 vs. 42		0.68	0.21-2.20	0.82	0.36-1.87	3.26	1.09-9.76
38 vs. 46		0.53	0.17-1.62	0.65	0.29-1.45	1.87	0.78-4.47
42 vs. 46		0.78	0.32-1.95	0.80	0.38-1.67	0.57	0.17-1.89

Source of variation	*P*-values		
Housing system	0.104	0.305	0.334
Age	0.511	0.545	0.077
Housing System×Age	0.622	0.568	0.838

[a, b] Means with different superscripts in the same column differ at $P \leq 0.05$. [A, B] Means with different superscripts in the same column differ at $P \leq 0.10$. [1] Ratio of the odds of hens having a specific keel bone issue over not having it. [2] Ratio of the odds of having keel bone problems for two treatment groups. [3] Confidence interval.

Table 2.6. Effect of housing system on tibia area, content and mineral density adjusted by BW at 47 wk of age

Housing system	Bone area (cm^2)	BMC (g)	BMD (g/cm^2)
Cage	3.310^{B}	0.754^{b}	0.231
Floor	4.558^{A}	1.096^{a}	0.238
	(0.394)	(0.095)	(0.007)
Source of variation		*P*-values	
Housing system	0.065	0.040	0.486

[a, b] Means with different superscripts in the same column differ at $P \leq 0.05$.
[A, B] Means with different superscripts in the same column differ at $P \leq 0.10$.
Values within parentheses are SEM.

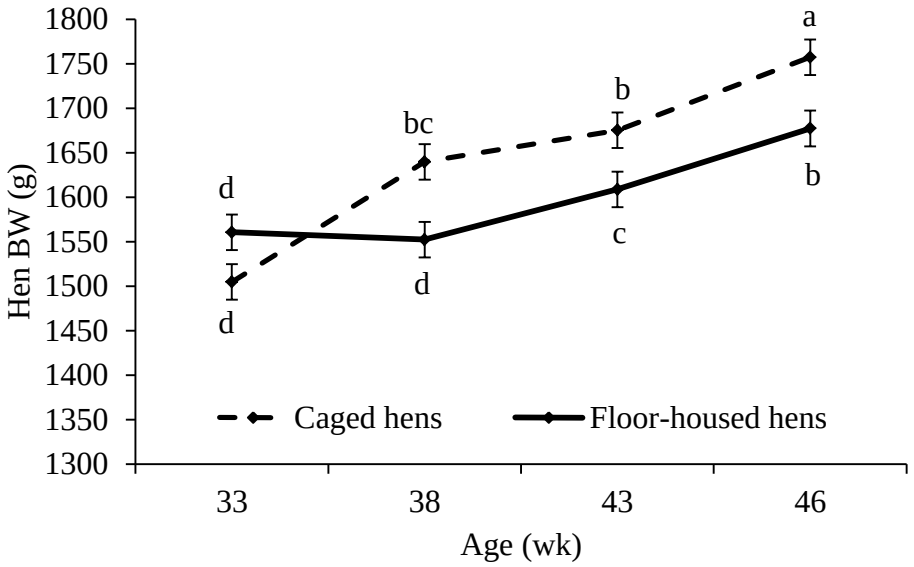

Figure 2.1. Interaction effect of housing system ($P < 0.0001$) on hen weight. [a - d] Means with different superscripts differ at $P \leq 0.05$. Bars indicate SEM.

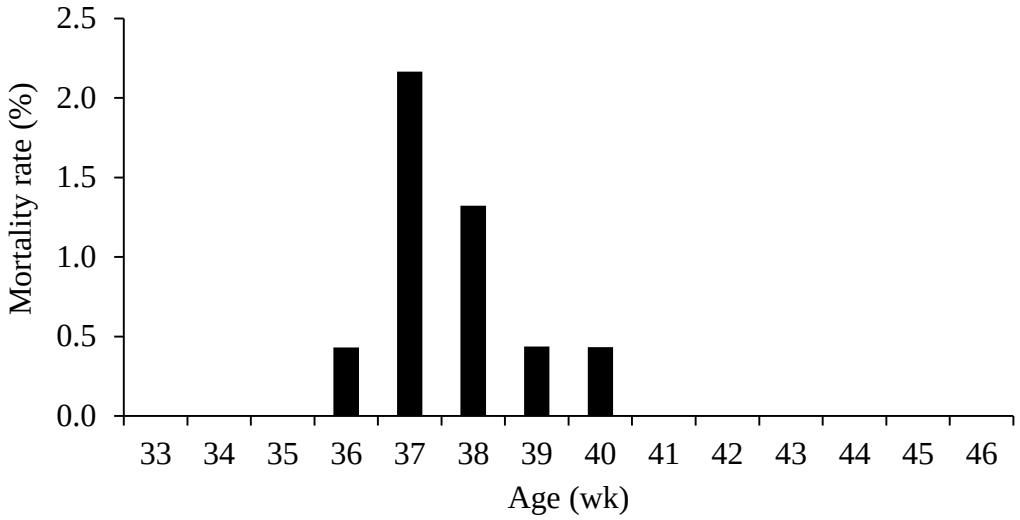

Figure 2.2. Hen mortality rate from 33 to 46 wk of age in floor pens.

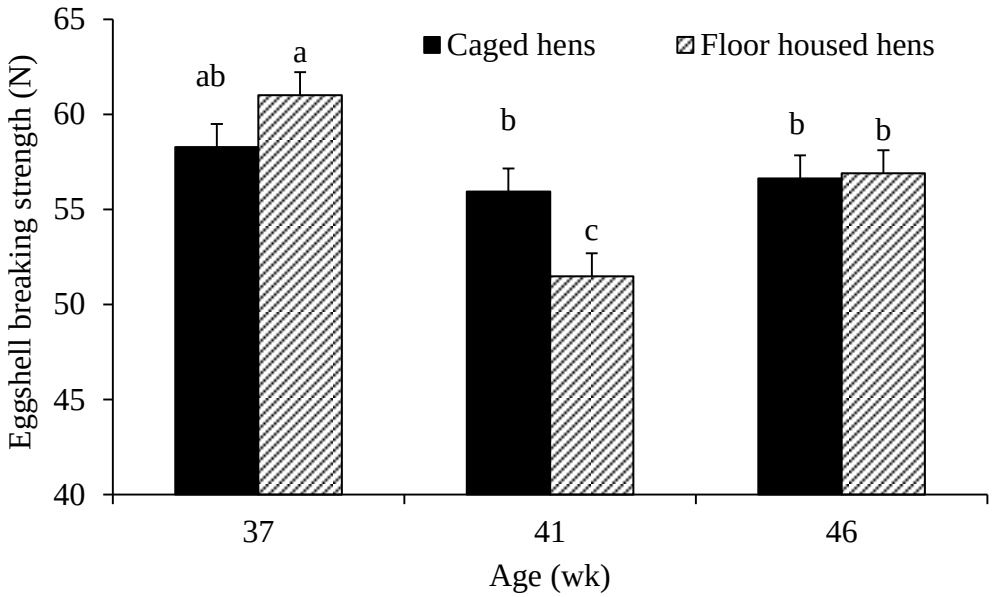

Figure 2.3. Interaction effect (P = 0.020) of housing system and age on eggshell breaking strength. [a, b] Means with different superscripts differ at $P \leq 0.05$. Bars indicate SEM.

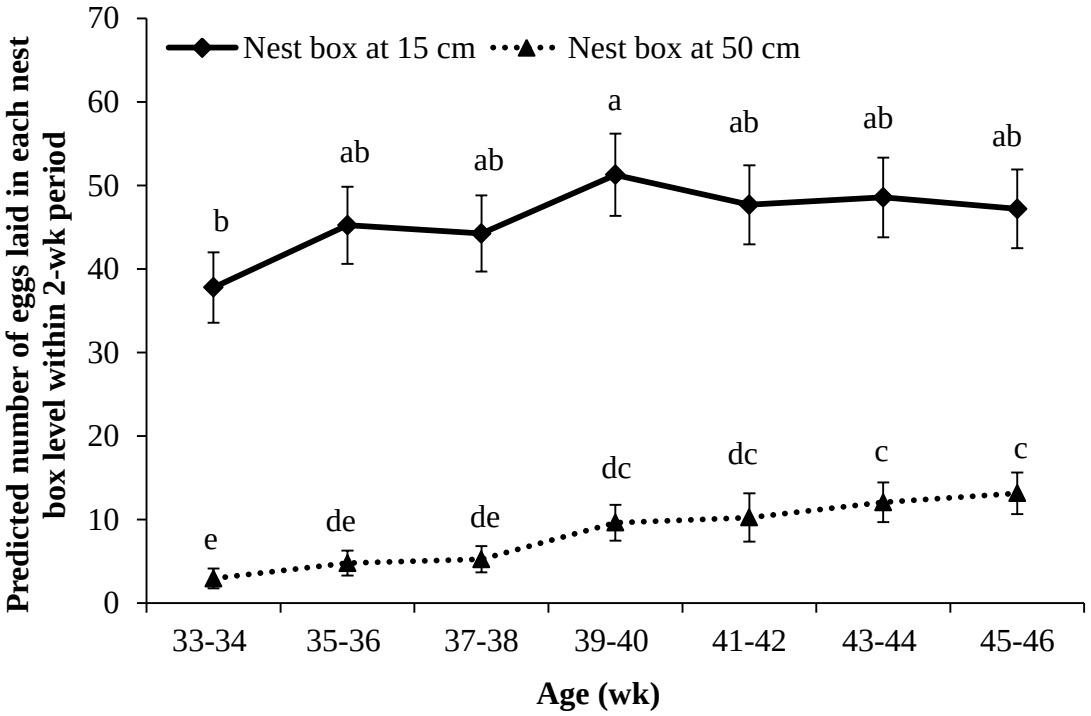

Figure. 2.4. Predicted number of eggs laid in each nest box level. [a - e] Means with different superscripts differ at $P \leq 0.05$. Bars indicate SEM.

CHAPTER 3. THE EFFECTS OF PARTICLE SIZE OF LIMESTONE FED TO WHITE OR BROWN PULLETS RAISED IN CONVENTIONAL CAGE OR AVIARY SYSTEMS ON PULLET GROWTH AND BONE INTEGRITY

3.1. INTRODUCTION

As there is more public awareness of animal welfare and increased legislation to protect animal well-being, the use of alternative housing systems for commercial egg layers is becoming more common. Although some studies have shown bone strength to improve in alternative housing systems because of greater hen activity, hens still have a high incidence of bone fractures. Bone fractures are not only welfare concerns but also could affect hen production (Nasr et al., 2013). In particular, keel bone fractures account for 90 % of bone breaks in alternative housing systems at end of lay (Wilkins et al., 2014).

Studies to improve skeletal health have focused on manipulation of environment and nutrition during the layer phase; however, at this phase it might be already too late to improve bone quality. Pullets reach their mature frame size by 12 wk of age and then formation of medullary bone takes place during the onset of egg production increasing up to 19 % of total body ash (Kwakkel et al., 1993). It is unlikely that pullets can mobilize from the skeleton enough calcium to properly undergo healing of a broken bone during bone development and medullary bone formation.

Therefore, it is important to study nutritional strategies in pullets to improve calcium digestion and absorption during the rearing phase in alternative housing systems. One of the nutritional strategies that has a major impact on calcium availability (Zhang and Coon, 1997) is the particle size of limestone. Limestone particle size in starter broiler diets has been extensively evaluated in the past (McNaughton, 1981; Guinotte and Nys et

al., 1991, Guinotte et al., 1995); however, because of the improvements in genetic selection towards high egg production and the widespread use of phytase in poultry diets, the reevaluation of particle size of limestone during the pullet rearing phase is needed.

Recent studies have indicated that a medium size rather than a fine particle size of limestone had beneficial effects on calcium utilization in pullets and broilers. Geraldo et al. (2004a) reported that a medium particle limestone (0.9 mm), but not a fine particle size (0.1 mm), included from 3 to 16 wk of age in pullets diets containing regular calcium levels increased tibia bone ash of cage pullets at the end of the pullet phase, but did not affect tibia bone ash at 5 (Geraldo et al., 2004b) or 12 (Geraldo et al., 2006) wk of age. In addition, broiler chicks fed a medium particle limestone (0.4 mm) in a diet containing a low level of available phosphorus (0.2 %) until 28 day of age tended to have higher tibia bone ash weight relative to tibia weight than when finer or larger particles were fed (Manangi and Coon, 2007).

Zhang and Coon (1997) reported that larger limestone particles (> 0.8 mm) accumulates in the gizzard and produces a high in vivo solubility that could potentially increase Ca retention. A larger limestone particle size could also slow down ionic Ca release into the gut improving calcium absorption. Manangi and Coon (2007) showed that limestone particle sizes interact with phytase activity in broilers. It is suggested that reduced calcium release in the gut prevents the formation of insoluble Ca-phytate phosphorus complexes (Selle and Ravindran, 2007) that cannot be hydrolyzed by phytase.

The amount of research that has been conducted to test limestone particle size for pullets is very low in cage systems and non-existent in aviary systems. In addition, effects

of limestone particle size on mineral status of broiler chicks might not extrapolate to pullets. On the other hand, little attention has been given to the effect of housing systems on the risk of keel bone injury during the pullet rearing phase. Therefore, the objective of this experiment was to evaluate the effects of two limestone particles; fine (0.4 mm) or a blend of fine and large particles (0.9 mm), fed from 7 to 16 wk of age to White or Brown pullets raised in aviary or cage housing systems on growth performance, bone mineral density, and incidence of keel bone deformities during the pullet rearing phase.

3.2. MATERIALS AND METHODS

Birds and husbandry

A total of 288 1-d-old pullets were randomly placed in 24 brooder battery cages (12 pullets/cage, 567 cm^2/bird) in a temperature controlled room. A total of 688 1-d-old pullets were also randomly housed in 8 wood shavings floor pens (86 pullets/floor pen, 598 cm^2/bird) in a tunnel-ventilated room. Lohmann Brown and Bovan White Leghorn chicks were intermingled in equal numbers in each cage or floor pen. At 5 wk of age, 288 cage pullets were moved to 48 pullet rearing cages (6 pullets/cage, 321 cm^2/bird) and 432 floor raised pullets were moved to 8 three tier aviary units (54 pullets/aviary unit, 1449 cm^2/bird) (Natura 60, Big Dutchman, Calveslage, Germany). At 5 wk of age, pullets were tagged in the neck skin (Swift tack, Heartland Animal Health, Fair Play, MO). At 10 wk of age, 256 cage pullets were transferred to 64 layer cages (4 pullets/cage, 510 cm^2/bird).

Pullet rearing cages and aviary units were located in the same temperature controlled room. Each cage provided one nipple drinker (4 birds/ nipple drinker) and a feeder trough (5.0 cm/bird). Each aviary provided 10 perches (20.0 cm/bird) at varying levels in the unit, access to floor area (626 cm^2/bird), two feeder troughs (4.4 cm/bird) at

the middle and lowest wire tier, and 6 and 2 nipples drinkers (7 birds/nipple drinker) at the highest and lowest wire tier, respectively.

To have similar number of pullets in experimental units, 6 contiguous cages (6 hens/cage) from 7 to 9 wk of age and 8 contiguous cages (4 hens/cage) from 10 to 17 wk of age constituted an experimental unit or cage group. Pullets were not beak trimmed. Feed and water were provided on an ad libitum basis. Management and vaccination practices were the same for cage and aviary system.

Pullets were given isocaloric and isonitrogenous experimental diets containing either fine limestone particles or a limestone blend of fine and medium particles (ILC Resources, Weeping Water, NE) from 7 to 17 wk of age. Average particle sizes were 0.431 and 0.879 mm for fine and blended limestone, respectively. Distribution of particles in fine and blend limestone are shown in Figure 3.1. Diet composition, calculated nutrient contents and analyzed total calcium and phosphorus are shown in Table 3.1. Diets were formulated using the recommendations for nutrients by Lohmann and Bovan Management Leghorn Guidelines. Calcium and phosphorus levels of experimental diets were similar; however, they were slightly higher in both experimental diets than formulated levels. Pullet diets were provided in mash form.

At 7 wk of age, half of the respective pullet populations from each aviary unit and each cage group were weighed as groups. Then, twenty six pullets (13 Brown and 13 White pullets) from each aviary and sixteen pullets (8 Brown and 8 White pullets) from each cage group were individually weighed at 11, 13, 15 and 17 wk of age to calculate BW coefficient of variation calculated by the standard deviation as percent of the mean. Feed intake was calculated by feed disappearance every two weeks except from the first

period that was from 7 to 10 wk of age. Because White and Brown pullets were intermingled in each aviary or cage, feed conversion ratio was calculated by dividing daily feed intake by average daily BWG of Brown and White pullets combined. All procedures were approved by the University of Nebraska-Lincoln Institute of Animal Care and Use Committee.

Bone examination

A sample of 176 randomly selected pullets (7 White and 7 Brown pullets/aviary unit and 4 White and 4 Brown birds/cage group) were scanned using a dual-emission x-ray absorptiometer (Norland Medical Systems, Fort Atkinson, WI) to determine in-vivo bone mineral density (BMD), bone mineral content (BMC) and bone area of right tibias including fibulas at 13 and 18 wk of age. Scanned pullets were identified by black leg zip ties. Non-anesthetized pullets were placed facing up on a foam device and restrained with Velcro straps around the neck, breast including the wings, and shanks for 12 minutes while the scan was taken (Hester et al., 2004). Pullets were individually weighed after the scan to use BW as a covariate in the data analysis.

At 16 wk of age, 336 randomly selected pullets (13 White and 13 Brown pullets/aviary unit and 7 White and 7 Brown pullets/cage group) were palpated to evaluate keel bone status (50 % of total number of pullets in each experimental unit). The palpation involved running 2 fingers down the side of the keel bone and feeling for degree of twists (curved keel bones), indentations (depressions with undefined edges) or fractures (sharp edges without palpable callus formation) (Clark et al., 2008).

Statistical analysis

Data were analyzed using a split plot 2 x 2 x 2 factorial design with GLIMMIX procedure (SAS, Cary, NC). Repeated measures analysis was also used for variables that were measured more than twice to determine changes in data through time. As repeated measures from the same subject are usually dependent, the measurements from the same subject over time might be correlated. To evaluate this correlation structure for each variable the following covariance patterns were tested: 1) compound symmetry, 2) autoregressive of order 1, 3) toeplitz, and 4) unstructured, using the AICC (AIC, Akaike information criterion, with a correction for finite sample sizes) to select the best fit for the model.

Housing system (aviary vs. cage system) and limestone particle size (fine vs. blended) were the main plots and strains (Brown vs. White) were the subplots. There was a total of 16 experimental units, 8 aviary units and 8 cage groups, resulting in a total of 4 replicates for each treatment combination. Statistical analysis of bone characteristics was conducted with BW as a covariate for each age.

Incidence of each keel bone deformity or injury was analyzed using a binomial logistic regression analysis because this variable was a designation of one of two possible outcomes (binary response), pullets having a specific keel bone issue or pullets having a normal keel bone without any keel bone damage. This analysis resulted in the generation of odds and odds ratio (Szumilas, 2010). Odds (o) are the probability (p) of having a specific keel bone issue over not having it ($1 - p$). Probability of having a specific keel bone issue can be calculated using odds following this formula: $p = o / (1 + o)$. While probabilities range from 0 to 1, odds range from 0 to positive infinity. Means were

separated using the LS means function and the SLICE option when applicable. Means were considered different at $P \leq 0.10$.

3.3. RESULTS

At placement, 1-d-old White pullets were heavier than 1-d-old Brown pullets ($P < 0.0001$) (Table 3.2). At 7 wk of age, the start of the feeding trial, Brown cage pullets had higher BW than Brown floor-raised pullets ($P < 0.0001$), whereas White pullets had similar BW in cages and floor pens ($P = 0.162$). Mortality was very low during the pullet feeding trial (7 to 17 wk of age); only two pullets out of 688 pullets (0.29 %) died in the aviary system at 14 and 17 wk of age for unknown reasons. Because of the low mortality, it was not possible to perform a statistical analysis.

Growth performance

Production performance of pullets during the pullet feeding trial was evaluated by obtaining body weight, body weight coefficient of variation, body weight gain, feed intake and feed conversion ratio. The *P*-values corresponding to overall production performance variables are shown in Table 3.3.

There was an interaction ($P = 0.082$) among housing system, strain and age for pullet BW (Figure 3.2), indicating that Brown pullets raised in aviaries had consistently lower BW than Brown pullets raised in cages from 11 to 17 wk of age, whereas White aviary pullets had lower BW than White cage pullets only at 17 wk of age. There was an interaction ($P = 0.072$) of strain, housing system and limestone particle size on overall pullet BW (Figure 3.3A). Brown pullets housed in cage systems had higher BW than Brown pullets housed in aviary systems fed either fine or a blend of fine and large

particles of limestone, whereas White pullets had similar BW regardless of housing system or limestone particle size used.

An interaction ($P < 0.0001$) between housing system and age indicated cage pullets had higher BW coefficient of variation than aviary pullets at only 11 and 13 wk of age (Figure 3.4). There was an interaction ($P = 0.065$) of strain, housing system and limestone particle size for overall BW coefficient of variation (Figure 3.3B).White cage pullets had higher BW coefficient of variation than Brown cage pullets when fed either the fine ($P = 0.029$) or the blended limestone ($P = 0.0003$). Brown pullets had higher BW coefficient of variation than White pullets when they were housed in the aviary system and fed the limestone blend ($P = 0.001$); however, strain did not affect ($P = 0.164$) BW coefficient of variation of pullets housed in aviary systems and fed fine limestone.

An interaction ($P = 0.084$) among strain, housing system, particle size of limestone and age was observed for BWG (Figure 3.5). This interaction showed that Brown aviary pullets fed the limestone blend had higher BWG than Brown aviary pullets fed the fine limestone from13 to 15 wk of age ($P = 0.098$, 8.0 vs. 4.9 g/d). For Brown cage pullets, the use of the limestone blend increased BWG from 13 to 15 wk of age ($P = 0.082$, 7.2 vs. 10.5 g/d). For White pullets fed a limestone blend, the use of aviary systems resulted in higher BWG from 7 to 11 wk of age ($P = 0.062$, 8.9 vs. 8.0 g/d) but lower BWG from 11 to 13 wk of age ($P = 0.017$, 13.7 vs. 10.2 g/d). For Brown pullets fed fine limestone, aviary systems had higher BWG than cage systems from 7 to 11 wk of age ($P = 0.036$, 12.2 vs. 11.1 g/d). For the first half of the pullet period, Brown pullets had higher BWG than White pullets in all treatment combinations with the exception of caged pullets fed the limestone blend. During the second half of the pullet period, Brown

pullets had higher BWG than White pullets only in the cage system and fed either the limestone blend ($P = 0.004$) from 13 to 15 wk of age or the fine limestone ($P = 0.011$) from 15 to 17 wk of age.

For feed intake and FCR, strain was not included in the statistical analysis as White and Brown pullets were raised in the same pen. There was no interaction among limestone particle size, housing system, and age for feed intake ($P = 0.390$). There was an interaction effect between housing system and age for feed intake ($P < 0.0001$) (Figure 3.6 A). Caged pullets had higher feed intake than aviary pullets from 7 to 10 wk ($P = 0.065$, 59 vs. 57 g/d), 11-12 wk ($P < 0.0001$, 64 vs. 58 g/d), 13-14 wk ($P < 0.0001$, 68 vs. 60 g/d) but lower feed intake than aviary pullets from 15 to 16 wk of age ($P = 0.011$, 65 vs. 68 g/d). Also, there was an interaction ($P = 0.047$) effect of housing system and limestone particle size for the overall feed intake, indicating that cage pullets fed the limestone blend had the highest feed consumption (66 g) in comparison with cage pullets fed the fine limestone (62 g) and aviary pullets fed either the blended (61 g) or the fine limestone (61 g). There was no interaction among strain, housing system and age for FCR ($P = 0.668$). There was an interaction effect between housing system and age for FCR ($P = 0.006$) (Figure 3.6B). Housing system did not affect FCR until the last period of the pullet rearing phase from 15 to 16 wk of age, at which age cage pullets had a better feed efficiency indicated by the lower FCR than aviary pullets ($P = 0.003$, 11.3 vs. 8.0). There was no interaction effect of housing system and limestone particle size for overall FCR ($P = 0.259$).

Tibia Bone characteristics

A covariance analysis with BW as a covariate was performed to evaluate tibia BMD, BMC, and bone area at 13 and 18 wk of age (Table 3.4). There was an interaction ($P = 0.023$) effect of housing system and strain at 13 wk of age (Figure 3.7). For 13-wk old Brown pullets, the use of aviary systems increased tibia bone mineral density compared to the use of cage systems ($P = 0.024$); however, for 13-wk old White pullets, housing system did not affect tibia bone mineral density ($P = 0.601$).

The utilization of the limestone blend increased tibia bone mineral density ($P = 0.034$) without affecting bone mineral content ($P = 0.177$) or bone area ($P = 0.743$) at 18 wk of age (Table 3.4). Pullets raised in aviary systems had greater tibia bone mineral density ($P = 0.061$) and area ($P = 0.001$) than pullets raised in cage systems at 18 wk of age. After BW adjustment, White pullets had higher tibia bone mineral density and content at 18 wk of age compared to Brown strain pullets ($P = 0.004$).

Keel bone health

As the highest order interaction ($P = 0.981$) among strain, housing system and limestone particle size was not evident for incidence of keel bone fractures, this interaction was removed from the statistical model. An interaction ($P = 0.073$) between housing system and limestone particle size indicated the odds of pullets fed fine limestone suffering keel bone fractures were 6.8 times the odds of pullets fed a limestone blend in cage systems ($P = 0.094$), while the odds of pullets fed either the fine or the blended limestone were similar in aviary systems ($P = 0.512$) (Table 3.5). Also, it was indicated that the odds of aviary pullets suffering keel bone fractures were 12.3 times the

odds of cage pullets when fed the blended limestone (P = 0.025) but not when fed the fine limestone (P = 0.556).

There was no interaction (P = 0.403) of housing system and limestone particle size for incidence of keel bone indentations. A interaction (P = 0.059) between strain and housing system indicated that the odds of White pullets having keel bone indentations were 3.0 times the odds of Brown pullets in aviary systems (P = 0.017), but not in cage systems (P = 0.642). Also, the odds of aviary pullets having keel bone indentations were 2.7 times the odds of cage pullets having keel bone indentations for White pullets (P = 0.049) but not for Brown (P = 0.456) pullets.

Regardless of strain and housing system, the use of the limestone blend resulted in lower odds of pullets having keel bone curvatures than the use of the fine limestone (0.14, confidence interval = 0.08 - 0.25 vs. 0.05, confidence interval = 0.02 - 0.11; P = 0.037). The odds of pullets fed the fine limestone displaying keel bone curvatures were 2.8 (confidence interval = 1.07 - 7.3) times the odds of pullets fed the limestone blend. An interaction (P = 0.069) between strain and housing system showed that the odds of White pullets having curved keel bones were 3.3 times the odds of Brown pullets having curved keel bones in aviary (P = 0.058) but not in cage (P = 0.427) systems. This interaction also indicated that the odds of aviary pullets having curved keel bones were less than the odds of cage pullets having curved keel bones for Brown pullets (P = 0.076) but not for White pullets (P = 0.416).

3.4. DISCUSSION

Growth performance

Cage pullets fed the blend limestone had the highest overall feed intake compared to cage pullets fed the fine limestone and compared to aviary pullets fed either type of limestone. This might be related to the increased growth rate from 13 to 15 wk of age of Brown pullets raised in cage systems and fed the blend rather than the fine limestone. Other experiments in conventional cage systems showed that White pullets fed a medium sized particle of limestone (0.899 mm) had lower feed intake than those fed a fine particle size of limestone (0.135 mm) from 3 to 12 wk (Geraldo et al., 2006) and from 3 to 16 wk (Geraldo et al., 2004). The author attributed the limestone effects on feed intake to the pullet preference for smaller particles to avoid discomfort after beak trimming that took place at 10 wk of age. Thus, disagreements in feed intake might be because pullets were not beak trimmed at any age in the present experiment.

Although experimental diets were formulated to have the same calcium level, pullets might have perceived a lower level of calcium because of the lower solubility and slower calcium release of larger limestone particles. Retention in the gizzard of particulate Ca larger than 0.8 mm has been demonstrated in birds by Zhang and Coon (1997). Higher feed intake might be an attempt to compensate the perception of low level of calcium during the day. Saunders-Blades et al. (2009) also reported greater feed consumption from 27 to 70 wk of age by laying hens fed a limestone blend of fine and coarse particle sizes (0.5 to > 4 mm) compared with fine limestone (< 0.42 mm). Similarly to our experiment, Geraldo et al. (2004, 2006) did not find effects of limestone particle size on FCR during the pullet rearing phase.

The evidence that strain effect on hen BW coefficient of variation in aviary systems was observed only when pullets were fed the limestone blend suggests that

individual pullet selection of specific limestone particle size (Classen and Scott, 1982; Olver and Malan, 2000) could have resulted in differences in nutrient intake on an individual pullet basis. None of previous pullet trials have evaluated the effect of particle size of limestone on BW uniformity.

Cage systems had heavier pullets during almost the entire pullet phase for Brown strain and during the end of the pullet phase for White strain. The provision of a more complex environment and larger floor area in aviary systems increased hen activity (Chapter 5) and might have resulted in greater energy expenditure and lower BW of aviary raised pullets. In a comparative study of conventional cage and free-range systems, cage pullets had also higher BW than free range pullets (Golden et al., 2012).

Cage pullets had higher feed intake than aviary pullets during most of the pullet rearing phase except from 15 to 16 wk of age, the period in which contrasting effects of housing system were observed. These contrasting effects could be associated with differences in onset of sexual maturity (Thiele and Pottgüter, 2008). Hurwitz et al. (1971) illustrated a sharp drop in feed intake four d before the first egg is laid. In fact, in a subsequent study, caged hens started to lay eggs two weeks earlier and had higher egg production during the peaking phase than aviary raised hens (Chapter 4).

Cage pullets had poorer BW uniformity than those housed in the aviary system during the first half of the pullet phase but improved BW uniformity by the end of the pullet phase. The improvement in BW uniformity might be a result of the increased floor space allowance from 321 cm^2/bird to 510 cm^2/bird at 10 wk of age. In a large scale experiment under commercial conditions, cage pullets had also 10 percent less BW uniformity (79 vs. 89 %) than floor raised pullets at 16 wk of age (Ortiz, 2011). However,

in a smaller experimental setting, cage systems and free-range system did not affect BW uniformity (Golden et al., 2012).

Bone health

Improvement of bone mineral density with the use of the limestone blend was observed only at 18 wk of age, probably indicating that this dietary strategy had more effect on medullary bone formation than on epiphyseal bone development. Our results were in accordance with Geraldo et al. (2006) who reported that limestone particle size did not affect tibia ash at 12 wk of age; however, at 16 wk of age, pullets fed a medium sized particle of limestone (0.899 mm) had higher tibia ash and calcium content than those fed a fine limestone (0.135 mm) at regular calcium levels.

Greater tibia BMD of pullets fed a limestone blend might be linked to keel bone integrity. For example, Wilkins et al. (2011) found a weak but significant negative relationship between prevalence of keel fractures and tibia ($R = -0.23$) and keel bone strength ($R = -0.28$) for hens raised in alternative housing systems. Toscano et al. (2013) reported a negative relationship between peak load of the keel bone measured ex vivo and tibia BMD (-0.83). In the present experiment, pullets fed the limestone blend had lower incidence of curved keel bones regardless of housing systems and strain, and lower keel bone fractures in cage systems.

The use of aviary systems increased pullet tibia BMD at 13 wk of age only for Brown pullets. At 18 wk of age, aviary raised pullets had higher tibia bone area but lower BMD than caged pullets. It has been previously reported that increased activity such as perching (Enneking et al., 2012) enhanced bone mineralization during the pullet rearing

phase. The larger BMD measured in pullets housed in cage systems relative to pullets in aviary systems at 18 wk of age might be a result of a faster sexual maturation and subsequent medullary bone formation of cage pullets as they had an earlier start of lay than aviary pullets (Chapter 4).

Aviary pullets had more severe keel bone problems and incidence of keel bone indentations especially for White pullets, and also higher incidence of keel bone fractures when fed a limestone blend compared to cage pullets. This could be related to the increased three dimensional space and provision of perches along with higher chances of hen interactions in aviary systems. Interestingly, Brown cage pullets had higher incidence of curved keel bones compared to Brown aviary pullets. The presence of curved keel bones in Brown cage pullets might be related to the lower BMD at 13 wk in the same pullet group. Also, higher BW of Brown cage pullets might have increased pressure on the keel bone while sitting compared to Brown aviary pullets.

White pullets had higher tibia BMD and BMC than Brown pullets at 18 wk of age after adjustment for BW. Strain and BW are confounding factors, especially when White and Brown strains are compared, because they have been selected to have different BW profiles when raised in similar conditions. Silversides et al. (2012) and Bennett et al. (2007) also highlighted the careful consideration of BW on the BMD analysis as BW is a major factor influencing BMD of load-bearing bones such as tibia.

In the present experiment, when values were not adjusted for BW, Brown pullets had higher BMD, BMC and tibial area compared to White pullets at 13 and 18 wk of age (data not shown), indicating that these observations were mainly related to differences in BW rather than strain effect by itself. Similar to these results, Neme et al. (2006) showed

that Brown pullets had higher ash deposition rate than White pullets from 4 to 18 wk of age.

White pullets displayed higher incidence of keel bone indentations and keel bone curvatures than Brown pullets in aviary systems. These results could be related to the reduced bone mineral density of White pullets because of its lower BW. To our knowledge, the effect of layer strain on specific keel bone incidence has not previously reported in cages or aviary units during the pullet phase.

3.5. CONCLUSIONS

The use of a limestone blend (0.9 mm) increased bone mineral status of pullets and alleviated incidence of keel bone curvatures, regardless of housing system. However, it ameliorated incidence of keel bone fractures only in cage systems. Further studies of either management practices or nutritional interventions are needed to reduce bone fractures in aviary systems during the pullet rearing phase, as they are serious welfare issues.

The higher BMD of cage pullets observed at the onset of egg production could be related to their earlier sexual maturation than aviary pullets. Although the more complex environment seemed to be the major causes of deteriorated keel bone integrity in aviary systems, different degrees of difficulty to catch pullets for management practices and data collection in cage and aviary system could have also influenced the effect of housing system on keel bone status during the pullet rearing phase. Finally, it is important to evaluate the carry-over effects of limestone particle size in pullet diets during the layer phase to draw final conclusions.

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Table 3.1. Diet compositions and calculated nutrient contents (as-fed basis)

Items	Pullet rearing phase		
	Grower (7-10 wk)	Developer (11-15 wk)	Prelay (16-17 wk)
Ingredients (%)			
Corn	66.52	69.88	63.47
Soybean meal	24.65	21.39	23.40
DDGS	5.00	5.00	5.00
Corn oil	0.28	0.47	1.02
Dicalcium phosphate	1.32	1.02	1.22
Limestone[1] (Fine vs. Blend)	1.26	1.35	5.02
Salt	0.38	0.38	0.38
Lysine	0.09	0.05	0.02
Methionine	0.08	0.07	0.07
Threonine	0.01	0.00	0.00
Vitamin & Mineral premix[2]	0.40	0.40	0.40
Amprolium	0.013	0.013	0.013
Calculated nutrients			
ME, kcal/kg	2990	3015	2900
Crude protein, %	18.25	16.17	16.62
Lysine, %	1.00	0.88	0.90
Methionine, %	0.39	0.36	0.36
Methionine+Cysteine,%	0.73	0.68	0.69
Tryptophan, %	0.21	0.19	0.20
Threonine, %	0.70	0.64	0.66
Calcium, %	1.00	0.95	2.40
Total analyzed calcium[3], %	1.12 / 1.19	1.19 / 1.20	3.10 / 3.34
Available phosphorus, %	0.48	0.41	0.45
Total analyzed phosphorus[3], %	0.73 / 0.82	0.57 / 0.56	0.70 / 0.69
Sodium, %	0.17	0.17	0.17

Ronozyme (500 FTU/g) was considered to release 0.1 % of Ca and P.

[1] Fine and blend limestone had an average particle size of 0.341 and 0.891 mm, respectively. ILC Resources, Weeping Water, NE.

[2]Vitamin and trace minerals provided the following per kilogram of feed: Vitamin A (retinyl acetate, 10,788 IU); vitamin D^3 (cholecalciferol, 4,381 IU); vitamin E (DL-α-tocopheryl acetate, 32 IU); vitamin K_3 (menadione dimethpyrimidinol, 4.0 mg); vitamin B_2 (riboflavin, 7.0 mg); vitamin B_5 (pantothenic acid, 9.0 mg); Vitamin B_3 (niacin, 46 mg); vitamin B7 (biotin, 93 mg); vitamin B_{12} (cobalamin, 11 mg); and choline ($C_5H_{14}ClNO$, 682 mg). Mn (MnO, 100 mg); Cu ($CuSO_4H_2O$, 7.5 mg); Fe ($FeSO_4H_2O$, 32 mg); Zn (ZnO, 73 mg); and Se (Na_2SeO_3, 0.24 mg).

[3] Total content of fine limestone-Diet/Total content of blend limestone-Diet. Method: AOAC 985.01: wet ash procedure that required mineral acids and heat.

Table 3.2. Initial BW and BW at the start of the feeding trial

Strain	Housing system	1d	7 wk
Brown		37.86[b]	725.07
White		39.68[a]	626.07
		(0.21)	(3.95)
Brown	Floor pens	-	700.89[b]
	Cage	-	749.25[a]
White	Floor pens	-	620.39[c]
	Cage	-	631.75[c]
		-	(5.59)
Source of variation		*P*-values	
Strain		< 0.0001	< 0.0001
Housing system		NA	< 0.0001
Housing system x strain		NA	0.003

NA: Not applicable.
[a-c] Means within the same column lacking a common superscript differ at $P \leq 0.05$.
Values in parentheses are SEM.

Table 3.3. The *P*-values of production performance during the pullet rearing phase

Source of variation	BW	BW CV[1]	BWG	Feed intake	FCR
Between subjects effects					
Strain (S)	< 0.0001	0.473	< 0.0001	NA	NA
Housing system (HS)	0.001	< 0.0001	0.001	0.001	0.004
Limestone particle size (LPS)	0.853	0.665	0.664	0.022	0.768
S×HS	< 0.0001	< 0.0001	0.029	NA	NA
LPS×S	0.979	0.884	0.580	NA	NA
LPS×HS	0.792	0.788	0.897	0.047	0.259
LPS× S×HS	0.075	0.065	0.139	NA	NA

[1] Body weight coefficient of variation calculated by dividing standard deviation by average BW. NA = Not applicable

Table 3.4. Limestone particle size, housing system and strain effects on tibia bone mineral density[1] (BMD), bone mineral content[1] (BMC) and area[1] at 13 and 18 wk of age

Treatments	13 wk			18 wk		
	BMD (g/cm^2)	BMC (g)	Area (cm^2)	BMD (g/cm^2)	BMC (g)	Area (cm^2)
Limestone particle size						
Fine	0.172	1.81	10.45	0.208[b]	2.57	12.31
Blend	0.174	1.82	10.40	0.215[a]	2.68	12.39
	(0.002)	(0.02)	(0.12)	(0.002)	(0.05)	(0.15)
Housing system						
Cage	0.171	1.79	10.41	0.215[a]	2.57	11.87[b]
Aviary	0.175	1.84	10.44	0.208[b]	2.68	12.83[a]
	(0.002)	(0.03)	(0.13)	(0.002)	(0.06)	(0.16)
Strain						
Brown	0.172	1.87	10.82	0.198[b]	2.40[b]	12.09
White	0.174	1.75	10.04	0.225[a]	2.85[a]	12.61
	(0.004)	(0.07)	(0.33)	(0.004)	(0.12)	(0.34)
Source of variation			*P*-values			
Limestone particle size (LPS)	0.424	0.875	0.780	0.034	0.177	0.743
Housing system (HS)	0.196	0.191	0.890	0.061	0.207	0.001
Strain (S)	0.779	0.370	0.229	0.004	0.062	0.435
HS×S	0.023	0.182	0.973	0.191	0.473	0.688
LPS×S	0.592	0.613	0.625	0.540	0.939	0.442
LPS×HS	0.610	0.434	0.792	0.173	0.421	0.848
LPS×HS×S	0.369	0.908	0.369	0.435	0.830	0.305
BW	0.096	0.025	0.159	0.0002	0.0003	0.0004

[1] Values were adjusted by BW recorded at the time of scan.

[a-c] Means within the same row lacking a common superscript differ ($P \leq 0.05$).

Values in parentheses are SEM.

Table 3.5. Odds and odds ratios of strain and housing system interaction and housing system and limestone particle size interaction for keel bone deformities at 16 wk of age

Treatments		Keel bone fractures		Keel bone indentations		Curved keel bones	
		Mean	CI[3]	Mean	CI[3]	Mean	CI[3]
Strain	Housing system			[1]Odds			
Brown	Cage	0.03	0.01-0.15	0.42[ab]	0.20-0.90	0.14	0.06-0.33
	Aviary	0.12	0.06-0.23	0.30[b]	0.15-0.57	0.04	0.01-0.11
White	Cage	0.05	0.01-0.18	0.33[b]	0.15-0.74	0.08	0.02-0.25
	Aviary	0.22	0.13-0.37	0.89[a]	0.51-1.55	0.13	0.07-0.25
Housing system	Limestone particle size						
Cage	Fine	0.10[ab]	0.04-0.25	0.39	0.18-0.84	0.23	0.12-0.44
	Blend	0.02[b]	0.00-0.12	0.36	0.16-0.79	0.05	0.01-0.17
Aviary	Fine	0.14[a]	0.08-0.26	0.71	0.40-1.27	0.09	0.04-0.21
	Blend	0.18[a]	0.10-0.33	0.37	0.20-0.69	0.06	0.02-0.14
Treatment comparisons				[2]Odds ratios			
Cage	White vs. Brown	1.58	0.37-6.82	0.79	0.34-1.79	0.57	0.13-2.42
Aviary	White vs. Brown	1.86	0.92-3.77	2.99	1.58-5.67	3.30	0.96-11.42
Brown	Aviary vs. cage	3.78	0.70-20.47	0.70	0.26-1.91	0.29	0.08-1.15
White	Aviary vs. cage	4.44	1.07-18.45	2.68	1.01-7.12	1.71	0.45-6.50
Aviary	Fine vs. Blend	0.76	0.33-1.77	1.91	0.81-4.51	1.59	0.46-5.48
Cages	Fine vs. Blend	6.83	0.70-66.45	1.10	0.36-3.31	4.91	1.15-21.02
Fine	Aviary vs. cages	1.37	0.46-4.04	1.81	0.69-4.75	0.40	0.14-1.17
Blend	Aviary vs. cages	12.26	1.40-107.48	1.04	0.38-2.86	1.25	0.26-6.07

Source of variation	*P*-values		
Strain (S)	0.258	0.208	0.506
Housing system (HS)	0.025	0.345	0.462
Limestone particle size (LPS)	0.177	0.270	0.037
HS×S	0.868	0.059	0.069
LPS×S	0.596	0.492	0.512
LPS×HS	0.073	0.403	0.234
LPS×HS×S	.	0.964	0.745

[1]Odds of having keel bone problems over not having them for each treatment group.
[2] Ratio of the odds of having keel bone problems for two treatment groups.
[3] Confidence interval.
[a-b] Means within a column lacking a common superscript differ ($P \leq 0.05$).

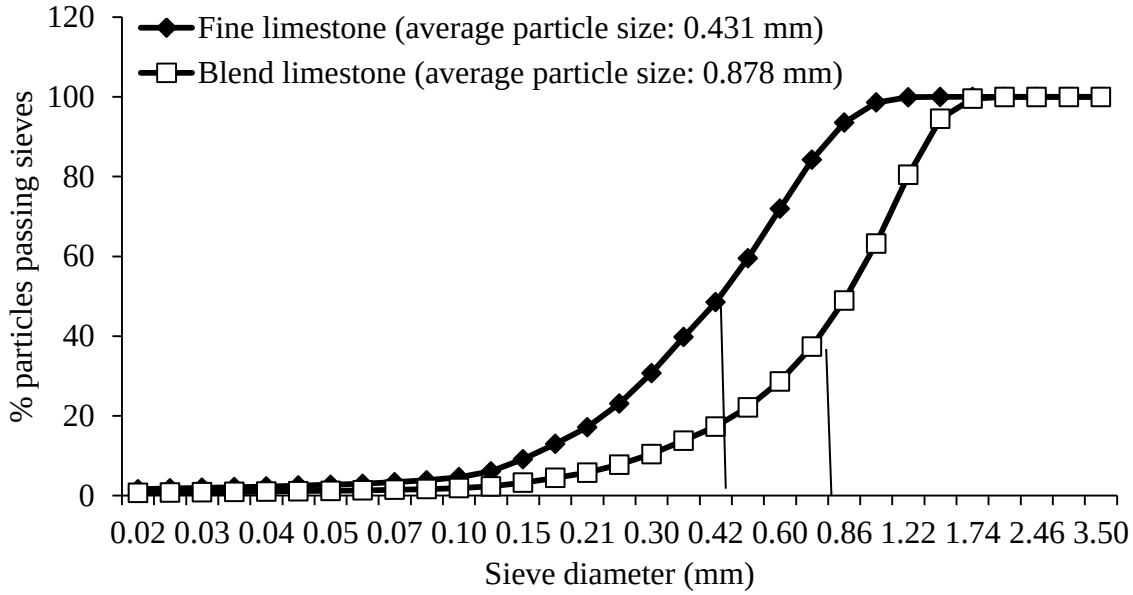

Figure 3.1. Particle size measurement using laser diffraction. From: ILC Resources (Weeping Water, NE) product specifications.

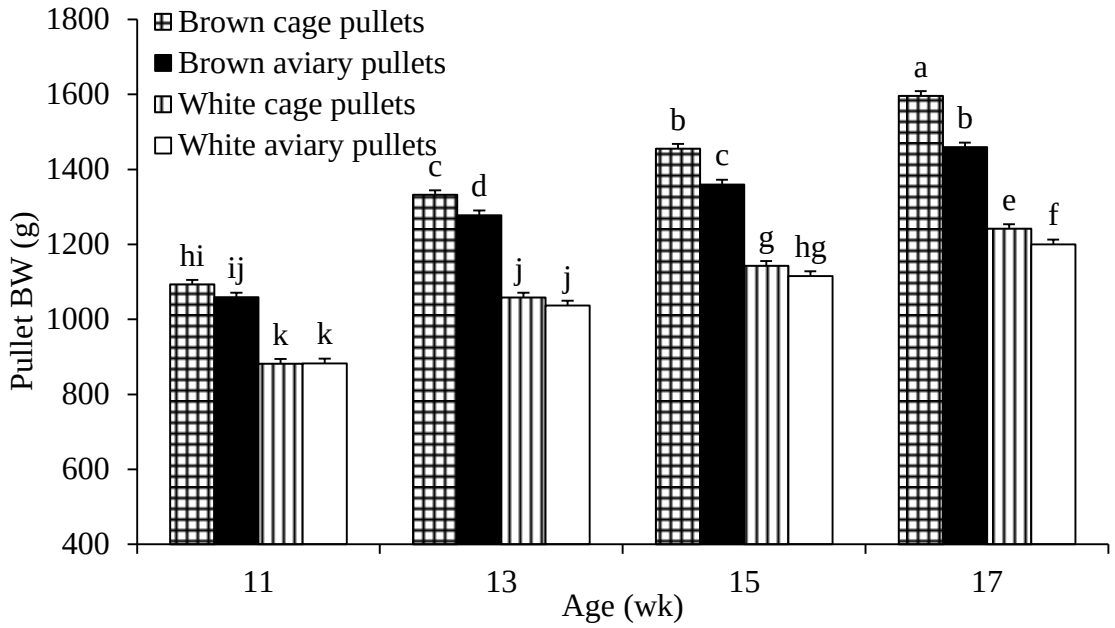

Figure 3.2. Interaction effect (P = 0.082) of strain, housing system and age on BW during the pullet rearing phase. [a-k] Means lacking a common superscript differ ($P \leq$ 0.05). Bars represent SEM.

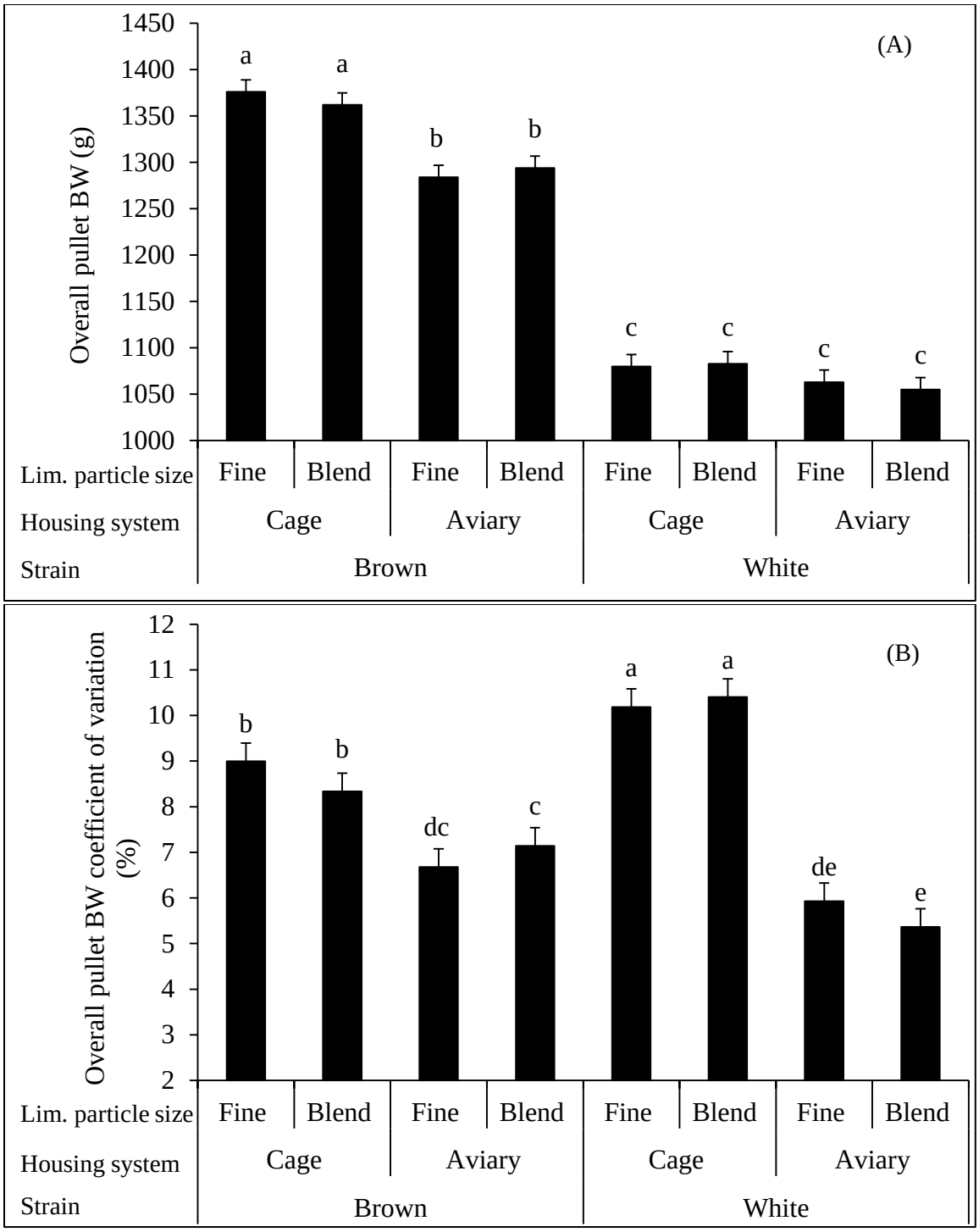

Figure 3.3. Interaction effect of strain, housing system and limestone particle size on overall BW (P = 0.075) (Panel A) and its coefficient of variation (P = 0.065) (Panel B) during the pullet rearing phase. [a-e] Means lacking a common superscript differ ($P \leq$ 0.05). Bars represent SEM.

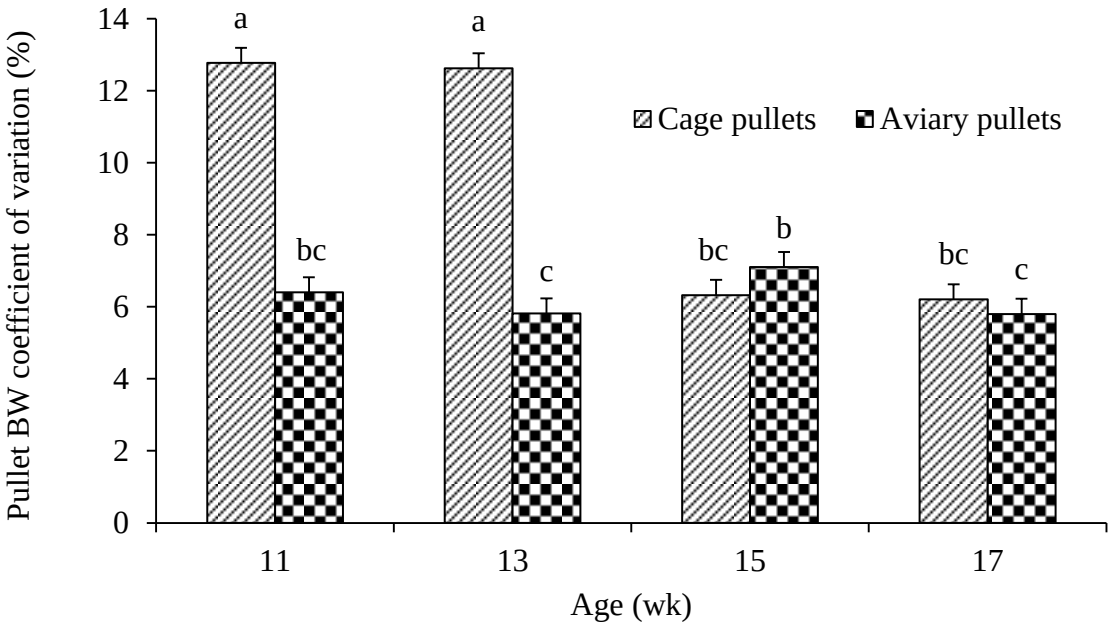

Figure 3.4. Interaction effect ($P < 0.0001$) of housing system and age on BW coefficient of variation during the pullet rearing phase. [a-c] Means lacking a common superscript differ ($P \leq 0.05$). Bars represent SEM.

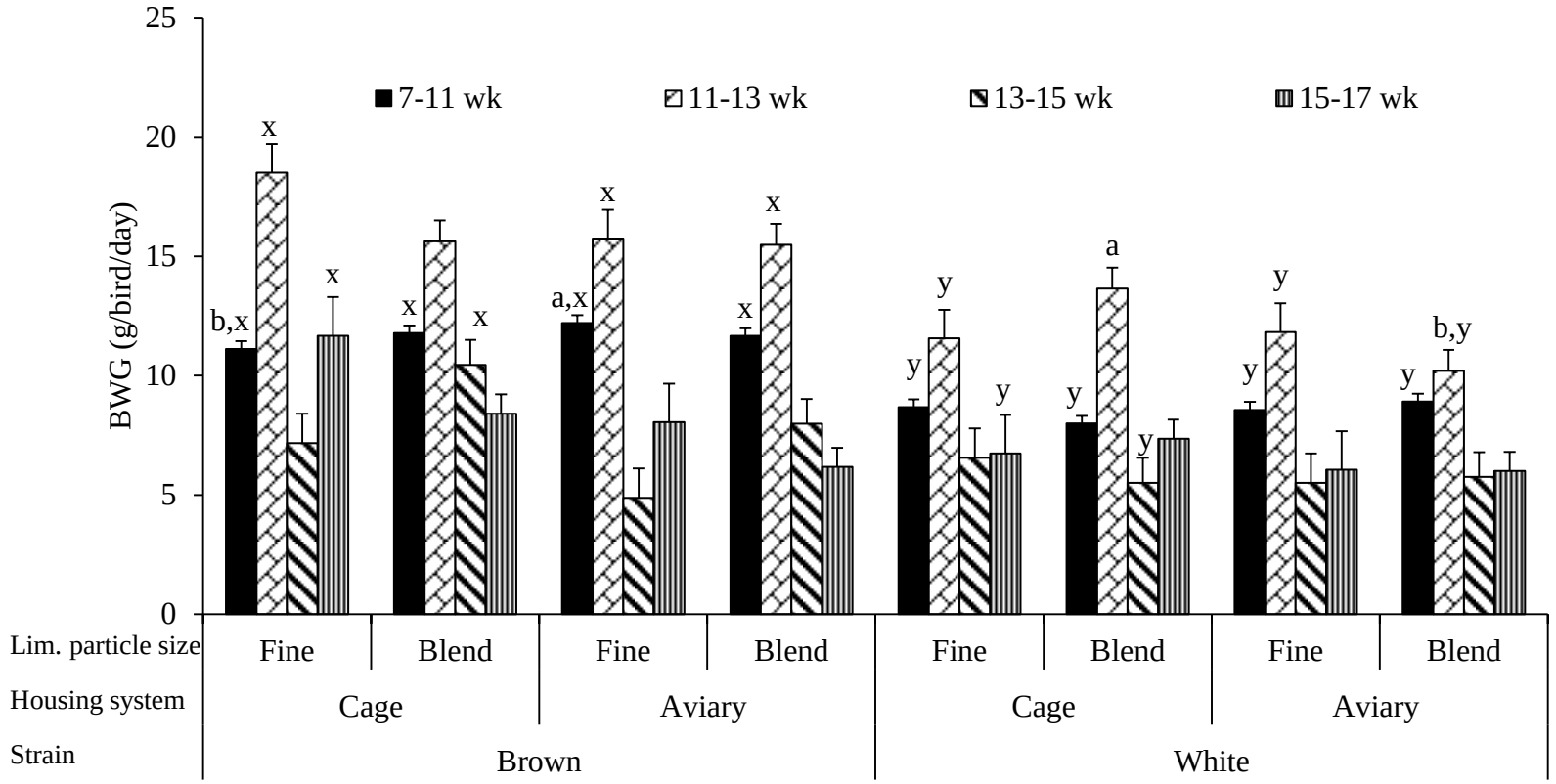

Figure 3.5. Interaction effect ($P = 0.084$) of strain, housing system and limestone particle size on BWG during the pullet rearing phase. [a,b] Means within age, strain, and limestone particle size having the same letter are not different ($P \leq 0.05$). [x,y] Means within age, housing system and limestone particle size having the same letter are not different ($P \leq 0.05$). Bars represent SEM.

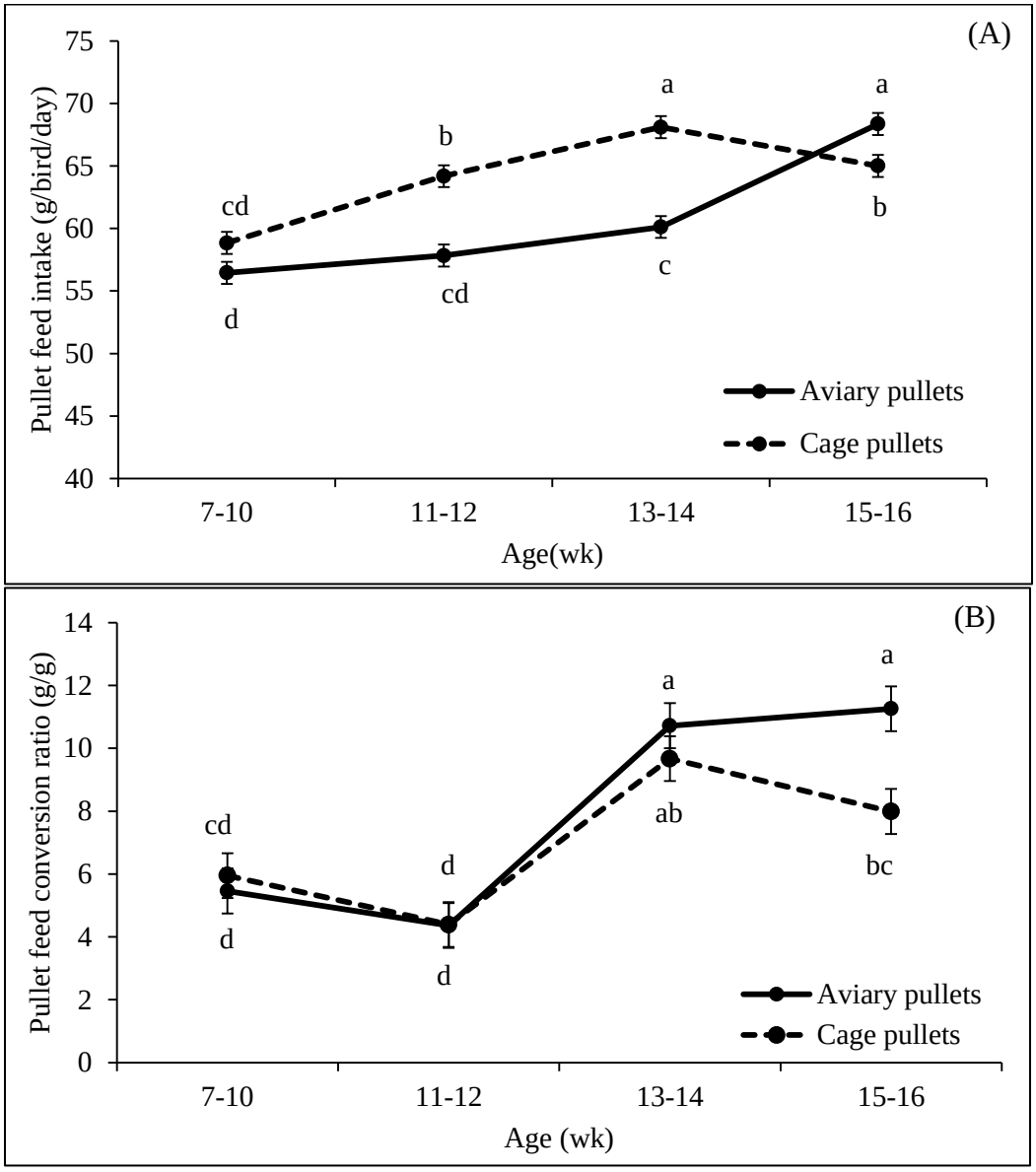

Figure 3.6. Effect of housing system over time on feed intake ($P < 0.0001$) (Panel A) and feed conversion ratio ($P = 0.0006$) (Panel B) during the pullet rearing phase. [a-d] Means lacking a common superscript differ ($P \leq 0.05$). Bars represent SEM.

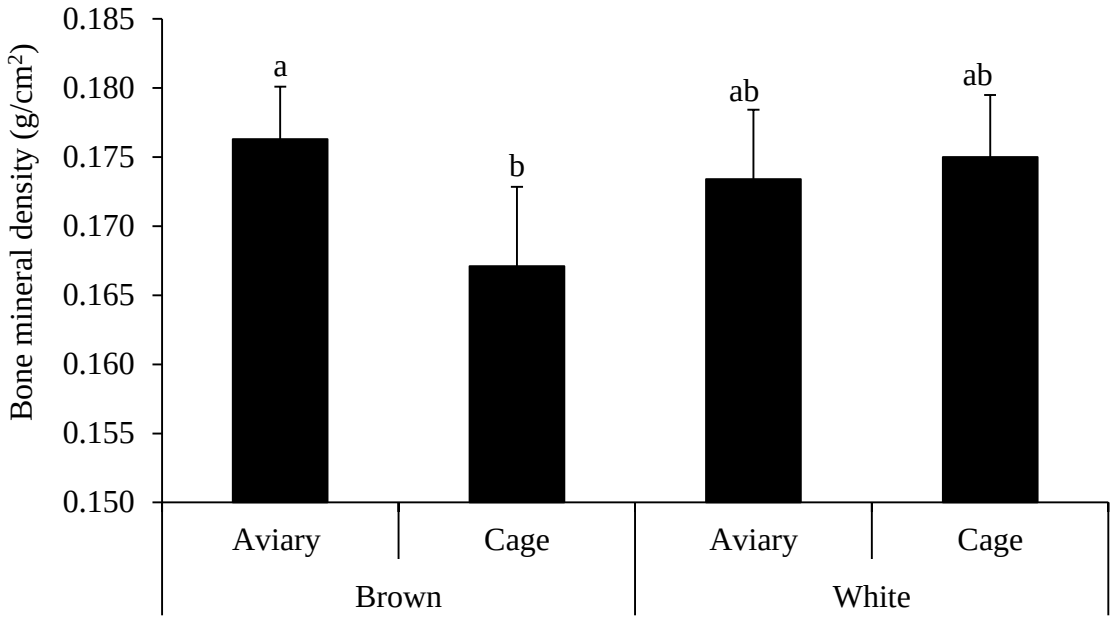

Figure 3.7. Interaction effect (P = 0.023) of housing system and strain on bone mineral density at 13 wk of age. [a,b] Means lacking a common superscript differ ($P \leq$ 0.05).

CHAPTER 4. CARRY OVER EFFECTS OF PULLET LIMESTONE PARTICLE SIZE ON EGG PRODUCTION, EGGSHELL QUALITY AND BONE HEALTH IN HENS RAISED IN CONVENTIONAL CAGE OR AVIARY SYSTEMS

4.1. INTRODUCTION

Alternative housing systems are becoming more common in the laying hen industry as awareness of hen welfare keeps increasing. Potential effects of pullet rearing and nutrition on skeletal integrity during the subsequent layer phase have not been extensively studied in conventional cage systems and even less in alternative housing systems. Past studies evaluating intervention strategies for improving bone integrity in laying hens have been done during the layer phase when the adult birds could already be experiencing osteoporosis. The modern day pullet experiences low feed intake at the onset of lay and has the genetic potential to rise quickly to peak production; substantial body reserves at the onset of production are indispensable to achieve satisfactory hen performance.

One of the major welfare issues facing the egg industry is the high incidence of keel bone fractures or deformities in alternative and cage housing systems. Some management practices during the pullet phase have been evaluated to improve bone quality of the laying hen. Access to perches during the pullet phase has improved bone mineralization at the end of the lay cycle but was not enough to reduce keel bone injuries (Hester et al., 2013). Also, a slow lighting program during the pullet rearing phase increased bone length and area suggesting a delay in bone growth plate closure at sexual maturity; however, bone mineralization was not affected at 66 wk of age (Hester et al., 2011).

Nutritional strategies during the pullet phase have also been evaluated in attempts to improve bone quality of the laying hen. An elevated Ca:P ratio in pullet and pre-lay diets increased femur breaking strength at the end of the layer phase without affecting pullet BW gain and pullet feed efficiency in cage systems (Fosnaught, 2009). Geraldo et al. (2006) reported that hens fed a medium limestone particle size as pullets had similar tibia bone ash at 30 wk of age compared to fine limestone. Therefore, there is some evidence that management practices and nutritional strategies during the pullet phase can have a positive carry-over effect during the lay cycle.

Measurement of eggshell characteristics along with bone traits is important for the evaluation of nutrition modifications given during the pullet phase. Bishop et al. (2010) recognized that there is a negative relationship between bone and eggshell strength. The author suggested that when bone quality is improved as a result of genetic selection, eggshell strength can deteriorate.

Our research team found that the inclusion of a blend of fine and large limestone particles increased bone mineral density (BMD) and alleviated keel bone injuries compared to fine limestone during the pullet phase (Chapter 3), the carry-over effects of this nutritional strategy need to be further evaluated during the layer phase. The objective of this experiment was to evaluate the nutritional strategies presented in Chapter 3, for Brown and White pullets raised in conventional cage and aviary systems on subsequent hen productivity, eggshell quality and bone health.

4.2. MATERIALS AND METHODS

Birds and Husbandry

Management practices and facilities used during the pullet rearing phase are described in Chapter 3. There were 50 hens (1565 cm^2/bird) in each of the 8 aviary units used (Natura 60, Big Dutchman, Calveslage, Germany) and 28 hens (510 cm^2/bird) in each of the 8 cage groups used (7 contiguous cages/cage group, 4 hens/cage). An empty cage was left between each cage group so that no cross feeding could take place. Lohmann Brown and Bovan White strain Leghorns were intermingled in equal numbers in each cage and aviary unit. Each conventional cage provided one nipple drinker (4 hens/ nipple drinker) and feed trough (5.0 cm/hen). Each aviary provided perches (22.0 cm/hen), nest area (120 cm/hen), access to woods shavings floor area (677 cm^2/hen), feeder troughs (4.8 cm/hen), and nipples drinkers (6 hens/nipple drinker). Cage and aviary system offered a sloping wire floor to facilitate roll out of eggs.

After two weeks of acclimatization, pullets had been given isocaloric and isonitrogenous experimental diets containing either fine (0.431 mm) limestone or a blend (0.879 mm) of fine and medium limestone particles (ILC Resources, Weeping Water, NE) from 7 to 17 wk of age (Table 3.1). In this study, hens received same layer diets throughout the trial. Diet composition and nutrient content used during the layer phase are shown in Table 4.1. Layer diets contained the same amount of limestone with 40 % from fine limestone and 60 % from bone and shell builder (2.486 mm) limestone (ILC Resources, Weeping Water, NE). Diets were formulated using the nutrient recommendations by Lohmann and Bovan Breeder Management Guidelines. Feed and water were provided ad libitum.

Feed intake was estimated by feed disappearance every two weeks. All eggs produced in one day were weighed at monthly intervals. Eggs were collected daily to obtain hen day (HD) and hen housed (HH) egg production from 17 to 52 wk of age. Egg mass was calculated by multiplying egg weight by HD egg production of the respective week. Because White and Brown hens were intermingled in each aviary or cage, feed conversion ratio was calculated by dividing daily feed intake by average daily egg mass of Brown and White eggs. All procedures were approved by the University of Nebraska-Lincoln Institute of Animal Care and Use Committee.

Eggshell characteristics and incidence of egg breakage

A total of 24 eggs (12 Brown and 12 White eggs) from each aviary unit and 14 eggs (7 Brown and 7 White eggs) from each group cage were sampled to evaluate eggshell breaking strength every 5 wk and eggshell percentage every 6 wk. Eggshell breaking strength, expressed as Newtons ($Kg \cdot m/s^2$), was tested using a Texture Analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY) measuring force to break the eggshell. Another group of eggs was weighed and broken open and albumen and yolk were removed to obtain eggshell weight. Eggshell percentage was expressed as a percentage of total initial egg weight. Cracked eggs were recorded daily from 18 to 52 wk of age at egg collection to evaluate overall incidence of eggshell breakage.

Bone measurements

A sample of 144 randomly selected laying hens (6 White and 6 Brown hens/aviary unit and 3 White and 3 Brown birds/cage group) were scanned using a dual-emission x-ray absorptiometer (Norland Medical Systems, Fort Atkinson, WI) to determine in vivo bone mineral density (BMD), bone mineral content (BMC) and bone

area of right tibias including fibulas at 36 and 52 wk of age. Hens were identified by black leg zip ties and used at both sampling times. Laying hens were individually weighed after x-ray scanning for use of BW as a covariate.

At 32 and 53 wk of age, a sample of 336 randomly selected hens (13 White and 13 Brown hens/aviary unit and 7 White and 7 Brown hens/cage group) were palpated to examine keel bone status (50 % of total number of hens in each experimental unit). The palpation involved running 2 fingers down the side of the keel bone and feeling for degree of twists (curvatures), indentations (depressions with undefined edges) or fractures (sharp edges without palpable callus formation) (Clark et al., 2008).

Statistical analysis

Data were analyzed using a split plot 2 x 2 x 2 factorial design with GLIMMIX procedure (SAS, Cary, NC). Repeated measures analysis was also used for variables that were measured two or more times to determine changes in data through time. As repeated measures from the same subject are usually dependent, the measurements from the same subject over time might be correlated. To evaluate this correlation structure for each variable the following covariance patterns were tested: 1) compound symmetry, 2) autoregressive of order 1, 3) toeplitz, and 4) unstructured, using the AICC (AIC, Akaike information criterion, with a correction for finite sample sizes) to select the best fit for the model. Statistical analysis of bone characteristics were conducted with BW at the time of scan as a covariate.

Housing system (aviary vs. cage system) and limestone particle size in pullet diets (fine vs. blend limestone particle size) were the main plots and strains (Brown vs. White) were the subplots. There was a total of 16 experimental units, 8 aviary units and 8 cage

group (7 contiguous cages/cage group) resulting in a total of 4 replicates for each treatment combination.

For statistical analysis, feed intake and FCR was evaluated monthly and HD and HH egg production were evaluated biweekly. Eggshell percentage were subjected to arcsine-square root transformation before statistical analysis. Incidence of each keel bone deformity or injury was analyzed using a binomial logistic regression analysis because this variable was a designation of one of two possible outcomes (binary response), pullets having a specific keel bone issue or pullets having a normal keel bone without any keel bone damage. This analysis resulted in the generation of odds and odds ratio (Szumilas, 2010). Odds (o) are the probability (p) of having a specific keel bone issue over not having it ($1 - p$). Probability of having a specific keel bone issue can be calculated using odds following this formula: $p = o / (1 + o)$. While probabilities range from 0 to 1, odds range from 0 to positive infinity. Means were separated using the LS means function and the SLICE option when applicable. Means were considered different at $P \leq 0.10$.

4.3. RESULTS

Hen production

There was no interaction ($P = 0.964$) of pullet limestone particle size and hen age on HD egg production (Figure 4.1A). On the other hand, there were interaction effects of housing system ($P = 0.0002$) and strain ($P < 0.0001$) with hen age on HD egg production. Cage hens had greater HD egg production than aviary hens throughout most of the trial except from 23 to 28 and 47 to 48 wk-of-age, periods in which housing effect was not apparent (Figure 4.1B). White hens had higher HD egg production than Brown hens

during most of the layer phase with the exception of a few time periods (Figure 4.1C). There was an interaction ($P = 0.004$) of pullet limestone particle size, housing system and strain on overall HD egg production (Table 4.2). Brown aviary hens fed a blend limestone as pullets had the lowest HD egg production. In every treatment combination, cage hens and White hens had higher overall HD egg production compared to their counterparts.

There was no interaction ($P = 0.278$) among pullet limestone particle size, housing system and strain on overall HH egg production (Table 4.2). But there was an interaction ($P = 0.01$) between limestone particle size in pullet diets and housing system on overall HH egg production (Table 4.2). Pullet limestone particle size did not influence overall HH egg production for Brown ($P = 0.42$) or White hens ($P = 0.99$), but Brown hens had lower HH egg production than White hens when fed the limestone blend as pullets (70.3 vs. 75.1 %, $P < 0.001$) and to a lesser degree when hens were fed fine limestone as pullets (72.73 vs. 75.10 %, $P = 0.025$).

There was an interaction ($P < 0.0001$) between housing system, strain, and age for HH egg production (Figure 4.2). Brown aviary hens had lower ($P < 0.10$) HH egg production than Brown cage hens for most of the layer phase with the exception of the start of lay and start of post peak egg production (17 to 18 and 23 to 30 wk of age). Housing system did not appear to affect HH egg production for White hens with very few exceptions (19 to 20 and 43 to 44 wk of age) during which White aviary hens had lower HH egg production than White cage hens. Also, this interaction demonstrated that Brown hens had lower ($P < 0.10$) HH egg production than White hens in aviary systems during the start and end of layer phase with few exceptions.

There was no interaction between pullet limestone particle size and hen age on egg weight (Figure 4.3A and Table 4.2).There was an interaction between age and housing system (P = 0.005) (Figure 4.3B) and strain (P = 0.09) (Figure 4.3C) for egg weight. From 23 to 35 wk of age, aviary and cage hens laid eggs of almost the same weight. However, there was a major (P = 0.03) increase in egg weight in aviary eggs from 35 to 38 wk of age, while weight of cage eggs did not change from this period (P = 0.75) onward. Brown hens produced consistently heavier eggs than White hens with the exception at 28 wk of age (P = 0.15) during strain effect was not apparent. There was an interaction (P = 0.0001) among limestone particle size in pullet diets, housing system and strain on overall egg weight (Table 4.2). For White hens, the blended limestone increased overall egg weights in cage systems (P = 0.001) but reduced overall egg weights in aviary systems (P = 0.01); whereas for Brown hens, particle size of limestone in pullet diets did not affect egg weight in aviary (P = 0.38) or cage systems (P = 0.14).

There was no effect of pullet limestone particle size, housing system, or their interaction on feed intake (P > 0.38) or FCR (P > 0.26). Cage hens had overall lower FCR than aviary hens (P = 0.06, 2.14 vs. 2.30).

Eggshell characteristics and incidence of egg breakage

There was an interaction effect of housing system (P < 0.0001) and strain (P < 0.0001) with age on eggshell weights and eggshell percentage (Table 4.3). Aviary hens laid eggs with heavier eggshells at 27, 37, and 52 (P = 0.11) wk of age (Figure 4.4A). Also, aviary hens laid eggs with greater eggshell percentages from 27 to 37 wk of age but lower eggshell percentages at 42 wk of age, without differences related to housing system afterwards (Figure 4.4C). White hens produced heavier eggshells from 27 to 32 wk of age

but lighter eggshells from 47 to 52 wk of age while strain did not affect eggshell weights in the middle of the layer phase (Figure 4.4B). Also, White hens had increased eggshell percentage from 27 to 37 wk of age but decreased eggshell percentage at 52 wk of age, while strain did not affect eggshell percentage from 42 to 47 wk of age (Figure 4.4D).

There was no interaction ($P = 0.28$) between pullet limestone particle size and hen age on eggshell weights. There was a main ($P = 0.05$) effect of pullet limestone particle size, demonstrating that hens fed the blended limestone as pullets laid eggs with greater overall eggshell weights than hens fed fine limestone (Table 4.3). There was an interaction ($P = 0.005$) between housing system and strain on overall eggshell weights. While Brown hens had higher overall eggshell weights than White hens in aviary systems ($P = 0.09$, 7.65 vs. 7.53 g, SEM = 0.04), Brown hens had lower overall eggshell weights than White hens in cage systems ($P = 0.02$, 7.21 vs. 7.38 g, SEM = 0.04). Also, this interaction indicated that aviary hens had higher overall eggshell weights than cage hens either from Brown ($P < 0.0001$) or White ($P = 0.01$) strains.

There was no interaction ($P = 0.196$) between pullet limestone particle size and hen age on eggshell percentage. There was an interaction ($P = 0.06$) effect among pullet limestone particle size, housing system and strain on overall eggshell percentage (Table 4.3). The use of the limestone blend as pullets only influenced overall egg percentage for Brown cage hens ($P = 0.06$) in which greater eggshell percentage was evident for hens fed blended limestone as pullets compared to hens fed fine limestone as pullets. Aviary hens had higher eggshell percentage than cage hens in almost all treatment combinations ($P < 0.03$) except for White hens fed fine limestone as pullets ($P = 0.49$). White hens had

higher eggshell percentage than Brown hens in all treatment combinations except for aviary hens fed fine limestone as pullets ($P = 0.21$).

There was no effect ($P = 0.803$) of pullet limestone particle size on overall eggshell breaking strength (Table 4.3). Aviary hens produced eggs with higher eggshell breaking strength than cage hens ($P = 0.003$). There was an interaction effect ($P = 0.05$) between strain with age on eggshell strength (Figure 4.5). Cage hens had a considerable drop in eggshell strength from 33 to 38 wk of age and a recovery at 43 wk of age, while aviary hens had a gradual increase of eggshell strength from 33 to 43 wk of age. This contributed to the elevated eggshell strength of eggs produced in aviary systems during most of the layer phase except for 33 and 53 wk of age. Eggshell strength in Brown hens decreased from 23 to 28 wk of age and remained low until 38 wk of age, bouncing back at 43 wk of age, while eggshell strength of White hens remained fairly constant from 23 to 48 wk of age. Eggshell strength in White eggs was consistently greater than that of Brown hens throughout the trial.

There was an interaction effect among pullet limestone particle size, housing system and strain on overall incidence of cracked eggs at the time of egg collection ($P = 0.066$) (Table 4.4 and 4.5). For Brown hens fed the blended limestone as pullets, the odds of observing cracked eggs at time of egg collection in aviary systems were 3.1 times the odds of observing cracked eggs in cage systems ($P = 0.039$). The odds of observing cracked eggs at time of egg collection from Brown hens were higher than the odds of observing cracked eggs from White hens when fed the limestone blend as pullets and housed in aviary system ($P = 0.001$) or fed fine limestone as pullets and housed in cage systems ($P = 0.006$).

Bone measurements

Bone characteristics were evaluated at 36 (Table 4.6) and 52 (Table 4.7) wk of age. Body weight was used as a covariate for all bone characteristics at both ages. There was no effect of pullet limestone particle size on any bone characteristics ($P > 0.10$) at 36 wk of age. Aviary housed hens had higher BMD ($P = 0.006$), BMC ($P < 0.0001$) and area ($P = 0.0002$) than caged hens at 36 wk of age. Brown hens had higher BMC ($P = 0.001$) and area ($P < 0.0001$) but had similar BMD ($P = 0.118$) to White hens at 36 wk of age.

There was an interaction ($P < 0.05$) effect among pullet limestone particle size, housing system and strain on all bone characteristics at 52 wk of age (Table 4.7). For caged White hens, the limestone blend fed as pullets reduced BMD ($P = 0.094$), BMC ($P = 0.005$), and area ($P = 0.004$) compared to the use of fine limestone at 52 wk of age. For White hens fed the blended limestone as pullets, the use of aviary system increased BMD ($P = 0.0006$), BMC ($P < 0.0001$) and area ($P = 0.004$) compared to the use of cage system at 52 wk of age. For Brown hens fed the fine limestone as pullets, the use of aviary systems increased BMC compared to the use of cage systems at 52 wk of age ($P = 0.064$). Aviary hens had increased bone area compared to cage hens in most of the treatment combinations ($P < 0.10$), with the exception of White hens fed the fine limestone as pullets at 52 wk of age ($P = 0.368$).

At 52 wk of age, Brown hens had lower BMD than White hens only when they were fed the limestone blend as pullets and housed in aviary systems ($P = 0.008$). At 52 wk of age, Brown hens had higher BMC than White hens when both had received the limestone blend as pullets and were housed in cage systems ($P = 0.0003$) and when both had been fed fine limestone as pullets and were housed in aviary systems ($P = 0.071$). At

52 wk of age, Brown hens had greater bone area than White hens in all treatment combinations ($P < 0.05$).

The incidence of keel bone fractures, indentations and twists are shown in Table 4.8 and 4.9. There was an interaction between particle size of limestone in pullet diets and age on keel bone indentations (Figure 4.6A). Lower odds of having keel bone indentations were apparent at 54 wk of age when hens had been fed blend limestone as pullets ($P = 0.02$), whereas there was no apparent effect at 32 wk of age. Also, it is evident that the odds of hens having keel bone indentations had a major increase from 32 to 54 wk of age, but the odds of hens having keel bone fractures ($P = 0.88$) and keel bone with curvatures ($P = 0.74$) remained relatively constant during this period. There was an interaction ($P = 0.09$) effect between housing system, strain, and age on the odds of having curved keel bones (Figure 4.6B). White hens had lower odds of having curved keel bones than Brown hens only in cage systems at 54 wk of age. Also, aviary hens had higher odds of having curved keel bones than cage hens at 32 ($P = 0.08$) and 54 ($P = 0.03$) wk of age for only White strain.

There was interaction ($P = 0.06$) between pullet limestone particle size and strain on overall odds of hens having keel bone fractures (Table 4.8 and 4.9).The overall odds of keel bone fractures in hens fed the fine limestone as pullets were 2.67 times the overall odds in hens fed the blended limestone as pullets for the White strain ($P = 0.03$) but not for the Brown strain ($P = 0.66$). The overall odds of White hens with keel bone fractures were 2.18 times the overall odds of Brown hens with keel bone fractures when fed fine ($P = 0.07$) but not blended limestone as pullets ($P = 0.36$). There was a main effect of housing system on the odds of having keel bone fractures ($P = 0.007$), keel bone

indentations ($P = 0.008$) and keel bone with curvatures ($P = 0.02$) indicating that these occurred more often in aviary than in cage systems.

4.4. DISCUSSION

Hen productive performance

The use of the limestone blend in pullet diets increased overall weights of eggs for White hens housed in cage system. This effect might be linked to observations from the pullet phase, indicating that the highest overall pullet feed intake was attained by pullets fed the limestone blend and raised in cage systems (Chapter 3). The lack of appreciable effect of pullet limestone particle size on feed intake and feed conversion ratio could be a consequence of the unavoidable exclusion of strain in our model; most of the effects of limestone particle size in pullet diets were apparent in interactions with strain by itself or along with housing system for most hen performance variables. In contrast, in aviary systems, the use of the limestone blend in pullet diets decreased egg weight in White hens and reduced hen-day egg production in Brown hens for unknown reasons.

Effects of housing systems on hen housed egg production were more evident for Brown hens than White hens because of the large difference in mortality in these two groups. Caged hens had higher egg production after adjustment for mortality than aviary hens especially during the onset of egg production indicating an earlier sexual maturity as well. Also, aviary hens had a higher feed conversion ratio than cage system indicating that aviary hens were converting feed less efficiently to egg mass. It is possible that feed intake used for feed conversion ratio calculation might be influenced by changes in feed wastage because of differences in feeder design between cage and aviary system.

Eggshell characteristics

Independent of housing system and strain, the use of the limestone blend fed as pullets increased overall eggshell weight. The limestone blend in pullet diets increased overall eggshell percentage in Brown cage hens. Greater tibia BMD achieved by pullets fed the blended limestone (Chapter 3) could indicate an enhanced medullary bone formation at the onset of sexual maturity. Medullary bone is more heavily calcified than cortical bone and serves as a labile source of calcium to support eggshell formation (Hurwitz, 1964). Although there were some improvements in eggshell traits because of the use of the limestone blend during the pullet phase, overall eggshell strength was not influenced.

Aviary hens produced heavier eggs with higher overall eggshell weights and percentage than cage hens and this in turn resulted in greater overall eggshell breaking strength. Similarly, Singh et al. (2009) and Vits et al. (2005) also found higher egg weights with hens housed in floor pens compared to hens in conventional cages. Tůmová et al. (2011) also reported that Bovan Brown hens housed in litter floor hens had higher eggshell strength than those in cages. Although aviary hens had stronger eggshells than cage hens, aviary systems had more eggshell breakage at egg collection than cage systems. This suggests that the improvement in eggshell quality was not sufficient for the egg to withstand more rough conditions in aviary systems.

One possible explanation for higher eggshell percentage and strength in the aviary system is that hens are free to recycle fecal calcium through coprophagia due the unrestricted access to litter floor (Harms et al., 1984) and this in turn could result in

higher Ca intake. Also, Neijat et al. (2011) reported that hens in furnished cages had improved calcium retention than those in conventional cages.

White hens had greater eggshell breaking strength indicating that these hens had stronger shells compared to Brown hens. As a result, higher percentage of egg breakage at time of collection was observed for Brown hens independent of housing system.

Bone health

Limestone particle size during the pullet phase did not affect any bone characteristics in hens at 36 wk of age. At 52 wk of age, lower BMD, BMC, and area were evident for White hens fed blended limestone as pullets in cage systems. The use of the limestone blend in pullet diets increased overall egg weight for White hens housed in cage system. Therefore, it is likely that this group had to mobilize more calcium and phosphorus from long bones such as tibia to sustain higher eggshell production. Geraldo et al. (2006) reported that hens fed limestone medium particles (0.9 mm) as pullets had similar tibia bone ash but higher bone calcium level at 30 wk of age compared to fine limestone fed as pullets (0.1 mm). Although responses were different from ours, this suggests that limestone particle size given during the pullet phase has an impact on bone quality during the layer phase.

Regardless of housing system and strain, the use of blended limestone in pullet diets decreased keel bone indentation incidence in hens at 54 wk of age. Regardless of hen age, the use of blended limestone fed as pullets reduced overall incidence of keel bone fractures only for White hens. Thus, the use of limestone blend (0.9 mm) rather than fine limestone (0.4 mm) in pullet diets has promising positive effects on keel bone

integrity late in the lay cycle. To our knowledge, this is the first experiment evaluating the effect of limestone particle size during the pullet phase on keel bone health in hens.

Aviary hens attained greater tibia BMD, BMC and area compared to cage hens at 36 wk of age. However, higher BMD of aviary hens at 52 wk of age was only observed for White hens fed the limestone blend as pullets. Increased exercise (Barnett et al., 1997, Hester et al., 2013), increased calcium retention (Neijat et al., 2011) and recycling of fecal calcium (Harms et al., 1984) in aviary systems are possible explanations of greater tibia BMD in this housing system. Silversides et al. (2012) also reported that tibia cortical density was higher in the floor pens than in cages but only for White hens. The author suggested that these strains had different behaviors, such as standing time in cages and usage of perches, in aviaries that could promote this strain and environment interaction.

After BW adjustment, White hens at 52 wk of age had higher BMD than Brown hens when both were fed the limestone blend as pullets and housed in aviary systems. However, when values were not adjusted for BW, Brown hens had higher BMD compared to White hens at 36 wk of age in both housing systems and at 52 wk of age in the cage system (data not shown), indicating that these observations were mainly related to differences in BW rather than strain effect by itself.

The higher overall incidence of keel bone fractures in White hens compared to Brown hens when fed fine rather than blend limestone in pullets diets might be related to their greater usage of perches (Chapter 6) as well as their higher degree of fearfulness observed by workers. Past research has showed that White hens had higher levels of fearfulness compared to Brown hens (Fraisse and Cockrem, 2006; De haas et al., 2013).

The higher incidence of curved keel bones for Brown hens in comparison with White hens in cage systems could have been caused by the higher load bearing forces on keel bones of Brown hens while sitting.

4.5. CONCLUSIONS

The provision of a limestone blend during the pullet rearing phase increased eggshell weight and reduced keel bone injuries during the layer phase. For White cage hens, the inclusion of the limestone blend from 7 to 17 wk of age increased egg weights and reduced overall tibia BMD at the end of the trial. However, the use of the limestone blend during the pullet phase had a deleterious effect on hen day production for Brown hens raised aviary systems. Further studies of pullet limestone particle size are needed especially for Brown hens raised in alternative housing systems.

4.6. REFERENCES

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Table 4.1. Diet composition and nutrient contents during the layer phase (as-fed basis)

Ingredients (%)	Peaking diet (17-33 wk)	Post Peak diet (33-52 wk)
Corn	54.33	53.36
Soybean meal	27.84	18.37
DDGS	5.00	15.00
Corn oil	2.05	2.07
Dicalcium phosphate	1.10	0.68
Fine limestone[1]	3.50	3.89
Shell and bone builder limestone[1]	5.25	5.83
Salt	0.38	0.34
Lysine	0.01	0.15
Methionine	0.13	0.10
Vitamin & Mineral premix	0.40[2]	0.20[3]
Amprolium	0.013	0.013
Calculated nutrients		
Metabolizable energy, kcal/kg	2800	2775
Crude protein, %	17.99	17.00
Methionine, %	0.47	0.39
Methionine+Cysteine, %	0.80	0.74
Lysine, %	1.00	0.90
Tryptophan, %	0.22	0.19
Threonine, %	0.80	0.66
Calcium, %	3.80	4.01
Total analyzed calcium[4], %	3.92	4.09
Available phosphorus, %	0.43	0.40
Total analyzed phosphorus[4], %	0.52	0.58
Sodium, %	0.17	0.18

Ronozyme (500 FTU/g) was considered to release 0.1 % of Ca and P.

[1] ILC Resources, Weeping Water, NE.

[2]Vitamin and trace minerals provided the following per kilogram of feed: Vitamin A (retinyl acetate, 10,788 IU); vitamin D_3 (cholecalciferol, 4,381 IU); vitamin E (DL-α-tocopheryl acetate, 32 IU); vitamin K_3 (menadione dimethpyrimidinol, 4.0 mg); vitamin B_2 (riboflavin, 7.0 mg); vitamin B_5 (pantothenic acid 9.0 mg); Vitamin B_3 (niacin, 46 mg); vitamin B7 (biotin, 93 mg); vitamin B_{12} (cobalamin, 11 mg); and choline ($C_5H_{14}ClNO$, 682 mg). Mn (MnO, 100 mg); Cu ($CuSO_4H_2O$, 7.5 mg); Fe ($FeSO_4H_2O$, 32 mg); Zn (ZnO, 73 mg); and Se (Na_2SeO_3, 0.24 mg).

[3]Vitamin and trace minerals provided the following per kilogram of feed: Vitamin A (retinyl acetate, 6,600 IU); vitamin D_3 (cholecalciferol, 2,805 IU); vitamin E (DL-α-tocopheryl acetate, 10 IU); vitamin K_3 (menadione dimethpyrimidinol, 2.0 mg); vitamin B_2 (riboflavin, 4.4 mg); vitamin B_5 (pantothenic acid, 6.6 mg); Vitamin B_3 (niacin, 24.2 mg); vitamin B_7 (biotin, 26 mg); vitamin B_{12} (cobalamin, 6 mg); and choline ($C_5H_{14}ClNO$, 322 mg). Mn (MnO, 54 mg); Cu ($CuSO_4H2O$, 6.6 mg); Fe ($FeSO_4H2O$, 22 mg); Zn (ZnO, 50 mg); and Se (Na_2SeO_3, 0.24 mg).

[4] Method: AOAC 985.01: wet ash procedure that required mineral acids and heat.

Table 4.2. Means and *P*-values of production performance during the layer phase

Strain	Housing system	Limestone particle size	Hen-day egg production (%)	Hen -housed egg production (%)	Egg weight[1] (g)
Brown	Cage	Fine	78.22^{cd}	77.85	61.77^{ab}
		Blend	80.28^{c}	79.32	60.99^{bc}
	Aviary	Fine	74.50^{e}	67.62	61.81^{ab}
		Blend	69.01^{f}	61.33	62.26^{a}
White	Cage	Fine	80.69^{ab}	75.25	58.95^{e}
		Blend	83.31^{a}	77.55	60.18^{dc}
	Aviary	Fine	77.12^{cd}	74.95	60.88^{c}
		Blend	78.20^{cd}	72.68	59.95^{d}
SEM			1.30	2.52	0.30
Source of variation			*P*-values		
Strain			< 0.0001	0.001	< 0.0001
Housing system			0.000	0.001	0.002
Limestone particle size			0.957	0.825	0.976
Strain×Housing system			0.002	0.002	0.631
Limestone particle size×strain			0.001	0.392	0.450
Limestone particle size×housing system			0.082	0.115	0.314
Limestone particle size×strain×housing system			0.004	0.970	0.0001

[a-f] Means within a column having the same letter are not different ($P \leq 0.05$).

Table 4.3. Interaction effect of strain, housing system and particle size of limestone on eggshell quality

Strain	Housing system	Particle size of limestone	Eggshell wt[1,3] (g)	Eggshell wt/egg wt[1,3] (%)	Eggshell strength[1,2] (N)
Brown			7.43	11.74[b]	54.97[b]
White			7.45	12.08[a]	59.17[a]
			(0.03)	(0.04)	(0.44)
	Cage		7.29	11.75[b]	55.65[c]
	Aviary		7.59	12.07[a]	58.48[a]
			(0.03)	(0.04)	(0.53)
		Fine	7.39[b]	11.88	56.97
		Blend	7.49[a]	11.94	57.17
			(0.03)	(0.04)	(0.53)
Brown	Cage	Fine	7.15	11.39[c]	54.02
		Blend	7.28	11.61[c]	53.10
	Aviary	Fine	7.62	11.98[b]	55.88
		Blend	7.67	11.99[b]	56.88
White	Cage	Fine	7.28	12.04[ab]	57.40
		Blend	7.47	11.97[b]	58.09
	Aviary	Fine	7.51	12.10[ab]	60.59
		Blend	7.55	12.21[a]	60.59
			(0.07)	(0.07)	(0.87)
Source of variation				*P*-values	
Strain (S)			0.641	< 0.0001	< 0.0001
Housing system (HS)			< 0.0001	< 0.0001	0.003
Limestone particle size (LPS)			0.049	0.247	0.803
S×HS			0.005	0.0005	0.980
LPS×S			0.818	0.322	0.728
LPS×HS			0.691	0.820	0.241
LPS×S×HS			0.138	0.062	0.691

[1] For aviary systems, each value represents the mean of 4 aviary units with an average of 12 eggs per aviary unit; for cage systems, each value represents the mean of 4 cage groups with an average of 7 eggs per cage group.
[2] Values represent the mean averaged over 7 ages from 23 to 53 wk of age at 5-wk intervals.
[3] Values represent the mean averaged over 6 ages from 27 to 52 wk of age at 5-wk intervals.
[a-c] Means within a column having the same small letter are not different ($P \leq 0.05$).
Values in parentheses are SEM.

Table 4.4. Odds[1] of observing cracked eggs at egg collection

Strain	Housing system	Limestone particle size	Cracked eggs at collection	
			Odds	CI
Brown	Aviary	Fine	0.0023[ab]	0.0010-0.0049
		Blend	0.0050[a]	0.0025-0.0099
	Cage	Fine	0.0023[ab]	0.0011-0.0049
		Blend	0.0016[bc]	0.0007-0.0036
White	Aviary	Fine	0.0015[bc]	0.0007-0.0034
		Blend	0.0016[bc]	0.0007-0.0034
	Cage	Fine	0.0006[c]	0.0002-0.0016
		Blend	0.0011[bc]	0.0004-0.0026

Source of variation	*P*-values
Limestone particle size (LPS)	0.436
Housing system (HS)	0.088
Strain (S)	0.0003
HS×LPS	0.696
LPS×S	0.753
LPS×S×HS	0.066

[1] Odds of finding cracked eggs during egg collection from 18 to 52 wk of age over not finding them.

[a-c] Means within a column having the same small letter are not different ($P \leq 0.05$).

Table 4.5. Odds ratio[1] of observing cracked eggs at egg collection

Strain	Housing system	Limestone particle size	Cracked eggs at collection		
			Odds ratio	CI of odds ratio	*P*-value
Brown	Aviary	Blend vs.Fine	2.21	0.79-6.21	0.121
Brown	Cage	Blend vs.Fine	0.70	0.23-2.10	0.491
White	Aviary	Blend vs.Fine	1.02	0.34-3.05	0.972
White	Cage	Blend vs.Fine	1.88	0.48-7.40	0.334
Brown vs. White	Aviary	Blend	3.17	1.85-5.44	0.001
Brown vs. White	Aviary	Fine	1.46	0.79-2.70	0.204
Brown vs. White	Cage	Blend	1.51	0.68-3.39	0.283
Brown vs. White	Cage	Fine	4.08	1.64-10.20	0.006
Brown	Aviary vs. Cage	Blend	3.09	1.07-8.89	0.039
White	Aviary vs. Cage	Blend	1.48	0.46-4.77	0.484
Brown	Aviary vs. Cage	Fine	0.98	0.33-2.87	0.962
White	Aviary vs. Cage	Fine	2.73	0.74-10.05	0.120

[1] Ratio of the odds of finding cracked eggs during egg collection for two treatment groups.

Table 4.6. Main effects of limestone particle size, housing system and strain on bone mineral density, content and area at 36 wk of age

Strain	Housing system	Limestone particle size	BMD[1] (g/cm^2)	BMC[1] (g)	Bone area[1] (cm^2)
Brown			0.266	3.68[a]	13.85[a]
White			0.277	3.21[b]	11.46[b]
	Cage		0.263[b]	3.21[b]	12.14[b]
	Aviary		0.280[a]	3.68[a]	13.16[a]
		Fine	0.269	3.42	12.65
		Blend	0.273	3.48	12.65
Pooled SEM			0.004	0.06	0.16
Source of variation				*P*-values	
Strain			0.118	0.001	< 0.0001
Housing system			0.006	< 0.0001	0.0002
Limestone particle size			0.482	0.483	0.990
Housing system×strain			0.603	0.623	0.629
Limestone particle size×strain			0.940	0.430	0.335
Limestone particle size×Housing system			0.686	0.979	0.583
Limestone particle size×Housing system×strain			0.761	0.508	0.294
BW			< 0.0001	0.0003	0.823

[a,b] Means within a column lacking a common superscript differ ($P \leq 0.05$).
[1] Values were adjusted by BW.

Table 4.7. Main and interaction effect of limestone particle size, housing system and strain on bone mineral density, content and area at 52 wk of age

Strain	Housing system	Limestone particle size	BMD[1] (g/cm^2)	BMC[1] (g)	Bone area[1] (cm^2)
Brown			0.275	3.55[a]	12.93[a]
White			0.287	3.18[b]	10.98[b]
	Cage		0.274[b]	3.15[b]	11.41[b]
	Aviary		0.288[a]	3.59[a]	12.51[a]
		Fine	0.283	3.42	12.08
		Blend	0.279	3.32	11.83
Pooled SEM of main effects			0.004	0.07	0.17
Brown	Cages	Fine	0.271[c]	3.37[ab]	12.38[bc]
		Blend	0.278[bc]	3.47[ab]	12.56[ab]
	Aviary	Fine	0.281[bc]	3.74[a]	13.43[a]
		Blend	0.269[c]	3.63[a]	13.35[a]
White	Cages	Fine	0.283[bc]	3.16[b]	11.06[d]
		Blend	0.265[c]	2.59[c]	9.62[e]
	Aviary	Fine	0.296[ab]	3.39[ab]	11.47[cd]
		Blend	0.305[a]	3.59[a]	11.79[bcd]
Pooled SEM of interaction effects			0.008	0.14	0.33
Source of variation				*P*-values	
Strain			0.072	0.005	< 0.0001
Housing system			0.043	0.0002	< 0.0001
Limestone particle size			0.551	0.307	0.272
Housing system×strain			0.020	0.067	0.414
Limestone particle size×strain			0.826	0.351	0.198
Limestone particle size×Housing system			0.760	0.142	0.113
Limestone particle size×Housing system×strain			0.038	0.013	0.037
BW			0.001	0.001	0.088

[a-e] Mean within the same column lacking a common superscript differ ($P \leq 0.05$)

[1] Values were adjusted by BW.

Table 4.8. The overall effect of particle size of limestone, housing system and strain on odds[1] of hens having fractured, indented, and curved keel bones during the layer phase

Strain	Housing System	Limestone particle size	Keel bone fractures		Keel bone indentations		Curved keel bones	
			Odds	CI[2]	Odds	CI[2]	Odds	CI[2]
Brown		Fine	0.23[ab]	0.11-0.46	0.60	0.35-1.06	0.46	0.29-0.72
		Blend	0.28[ab]	0.15-0.51	0.40	0.24-0.67	0.26	0.15-0.47
White		Fine	0.50[a]	0.30-0.82	0.64	0.36-1.13	0.27	0.15-0.49
		Blend	0.19[b]	0.10-0.36	0.65	0.41-1.03	0.27	0.16-0.48
Brown	Cage		0.13	0.06-0.29	0.40	0.22-0.75	0.31[ab]	0.17-0.58
	Aviary		0.50	0.33-0.75	0.60	0.39-0.92	0.39[a]	0.26-0.58
White	Cage		0.24	0.12-0.48	0.36	0.20-0.68	0.15[b]	0.07-0.31
	Aviary		0.39	0.25-0.61	1.13	0.76-1.70	0.49[a]	0.33-0.71
	Cage		0.17[b]	0.10-0.30	0.38[b]	0.25-0.59	0.22[b]	0.14-0.35
	Aviary		0.44[a]	0.32-0.60	0.83[a]	0.61-1.11	0.44[a]	0.33-0.57

Source of variation	*P*-values		
Strain (S)	0.512	0.297	0.357
Housing system (HS)	0.007	0.008	0.018
Limestone particle size (LPS)	0.191	0.439	0.302
Strain×Housing system	0.151	0.160	0.090
Limestone particle size×Strain	0.062	0.402	0.299
LPS× HS	0.858	0.281	0.684
LPS×S×HS	0.517	0.377	0.406

[a,b] Means within a column lacking a common superscript differ ($P \leq 0.05$).

[1]Odds of having keel bone problems over not having it for each treatment group.

[2] Confidence interval

Table 4.9. Interaction effect ($P = 0.062$) of strain and limestone particle size on odds ratios[1] of keel bone fractures and interaction effect ($P = 0.090$) of strain and housing system on odds ratio of curved keel bones during the layer phase

Strain	Housing System	Limestone particle size	Odds ratios		
			Mean	CI[2]	*P*-value
Keel bone fractures					
Brown		Fine vs. Blend	0.83	0.33-2.08	0.660
White		Fine vs. Blend	2.67	1.16-6.13	0.025
White vs. Brown		Fine	2.18	0.93-5.10	0.069
White vs. Brown		Blend	0.68	0.27-1.67	0.363
Curved keel bones					
Brown	Aviary vs. Cage		1.26	0.60-2.64	0.009
White	Aviary vs. Cage		3.20	1.41-7.25	0.510
White vs. Brown	Aviary		1.25	0.72-2.17	0.393
White vs. Brown	Cage		0.49	0.19-1.28	0.132

[1] Ratio of the odds of having keel bone problems for two treatment groups.
[2] Confidence interval

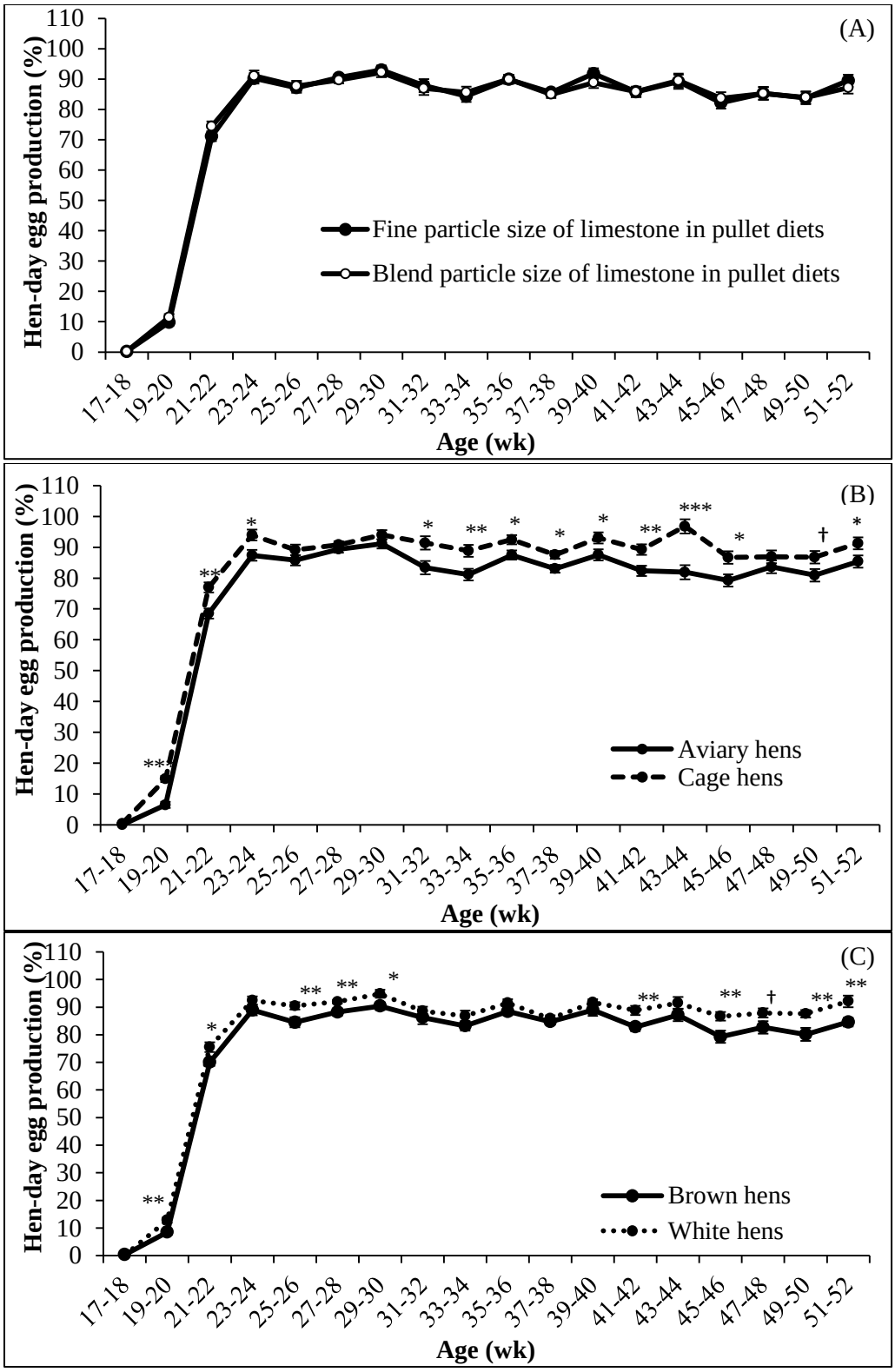

Figure 4.1. Effect of particle size of limestone in pullet diets ($P = 0.964$) (Panel A), housing system ($P = 0.0002$) (Panel B) and strain ($P < 0.0001$) (Panel C) on hen day egg production over time. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and †$P \leq 0.10$. Bars represent SEM.

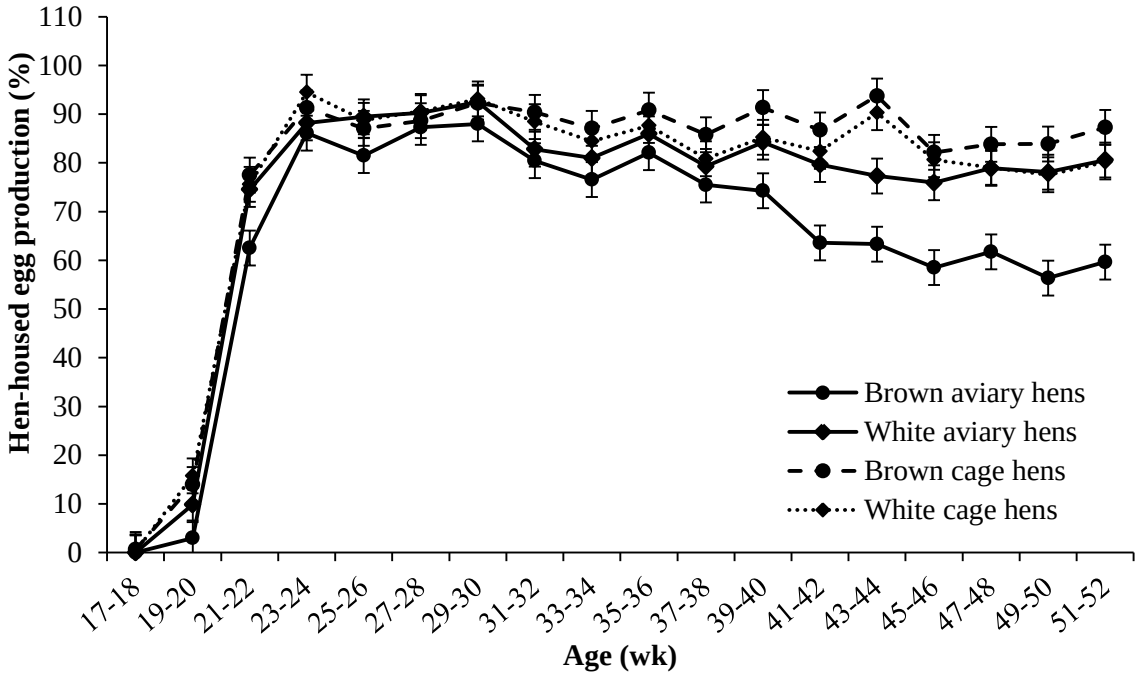

Figure 4.2. Interaction effect ($P < 0.0001$) of housing system and strain over time. Bars represent SEM.

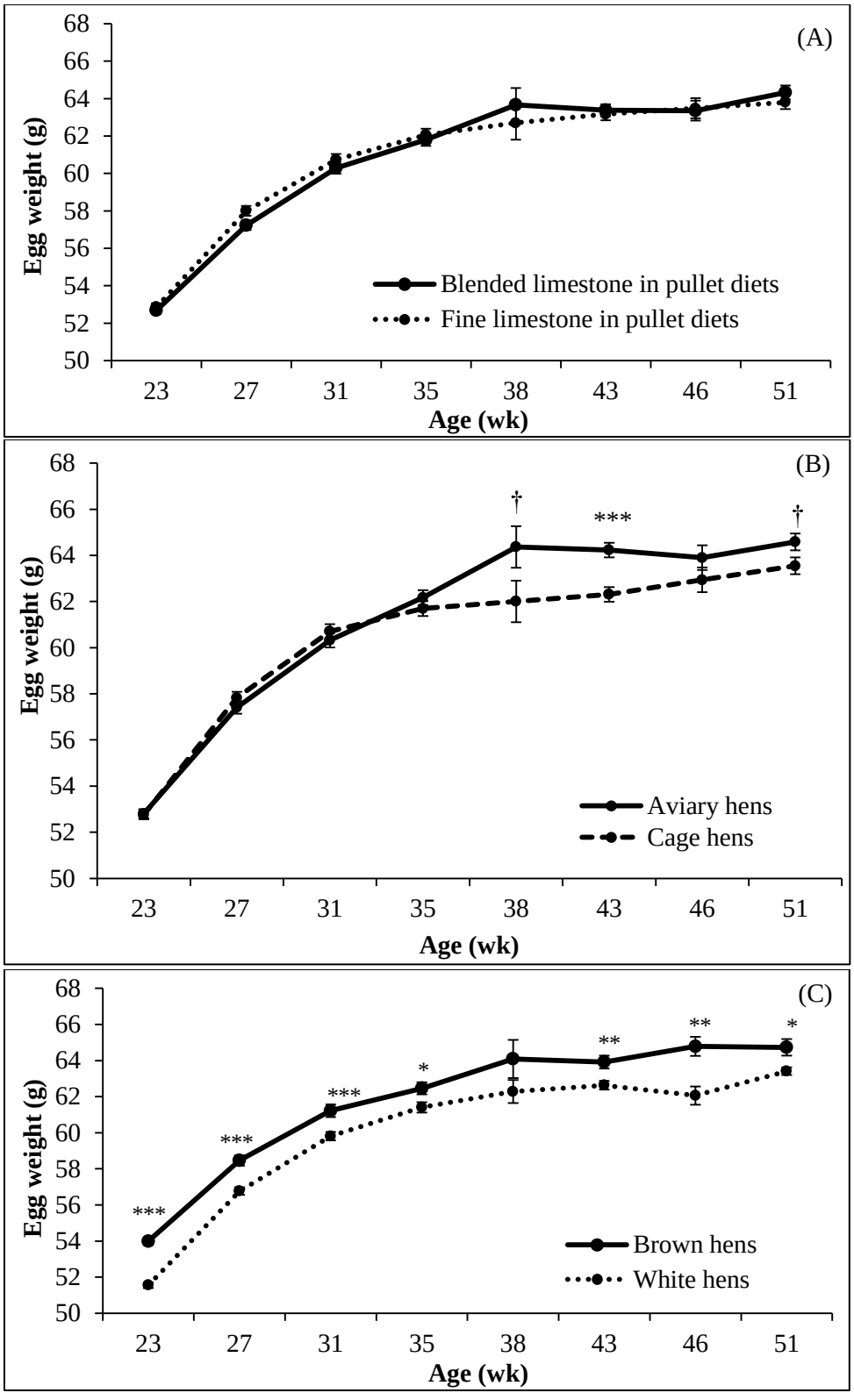

Figure 4.3. Effect of limestone particle size ($P = 0.562$), housing system ($P = 0.005$), and strain ($P = 0.086$) over time on egg weight from 23 to 51 wk of age. Means were separated by *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and †$P \leq 0.10$ within each age. Bars represent SEM.

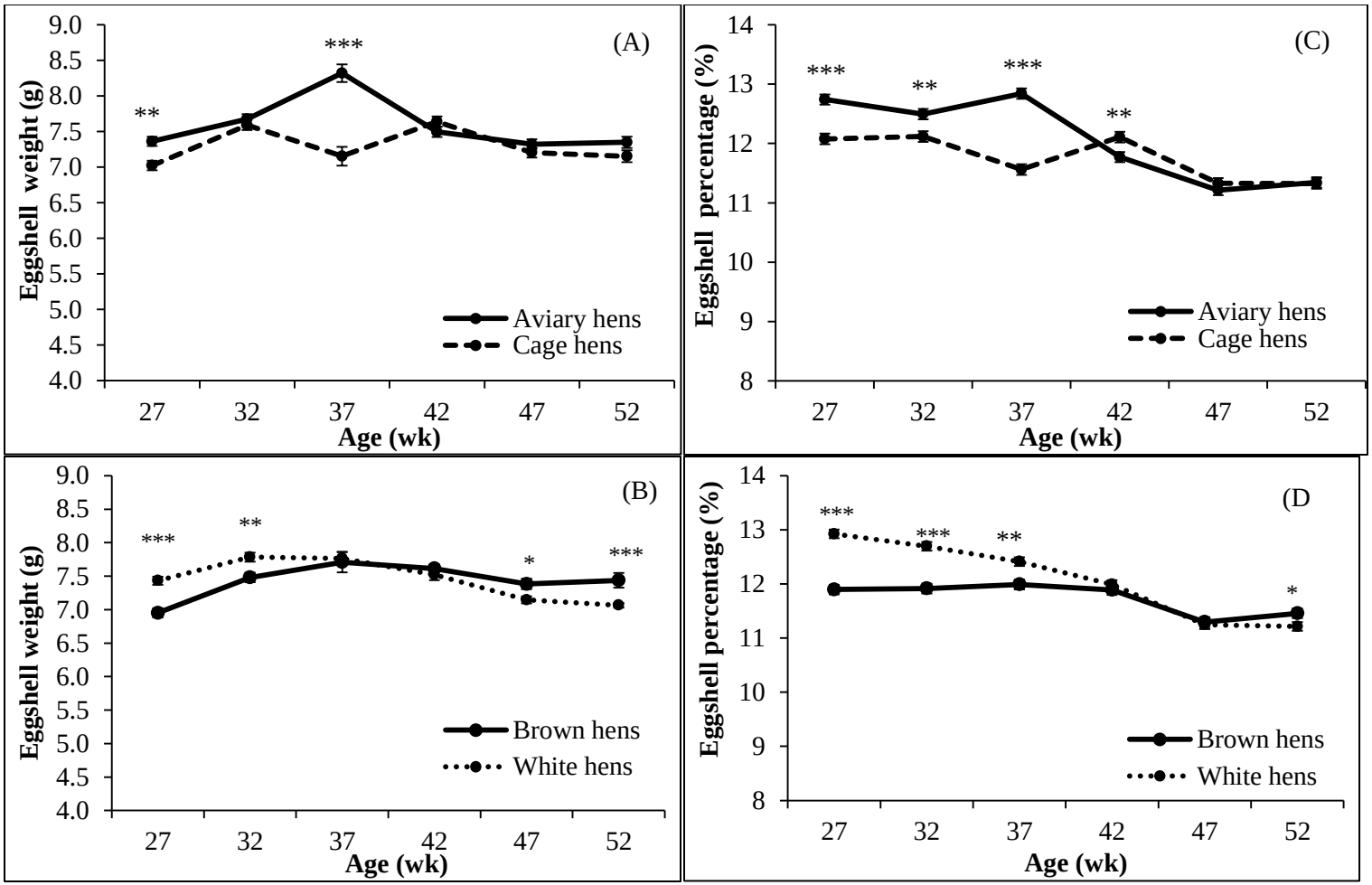

Figure 4.4. Effect of housing system ($P < 0.0001$) (Panel A) and strain ($P < 0.0001$) (Panel B) over time on eggshell weights and effect of housing system ($P < 0.0001$) (Panel C) and strain ($P < 0.0001$) (Panel D) on eggshell percentage. Means were separated by *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and †$P \leq 0.10$ within each age. Bars represent SEM.

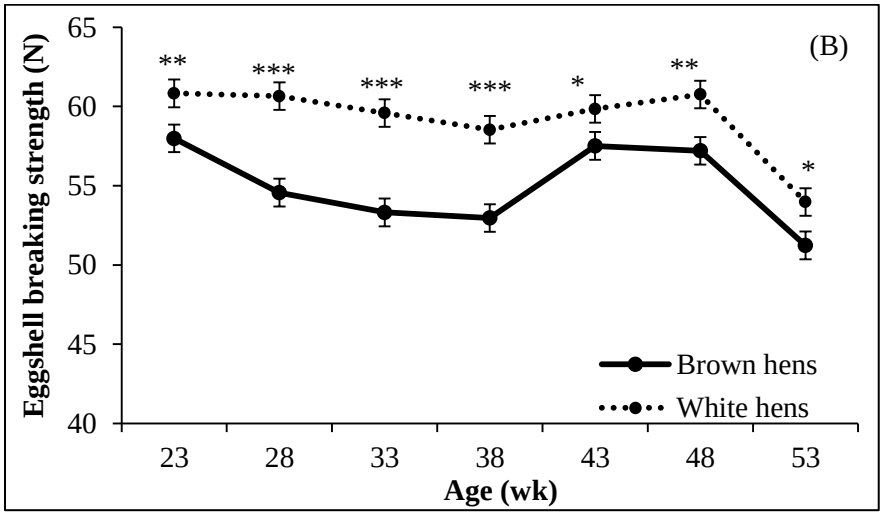

Figure 4.5. Interaction effect ($P = 0.050$) of strain and age on eggshell breaking strength. Means were separated by $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$ within each age. Bars represent SEM.

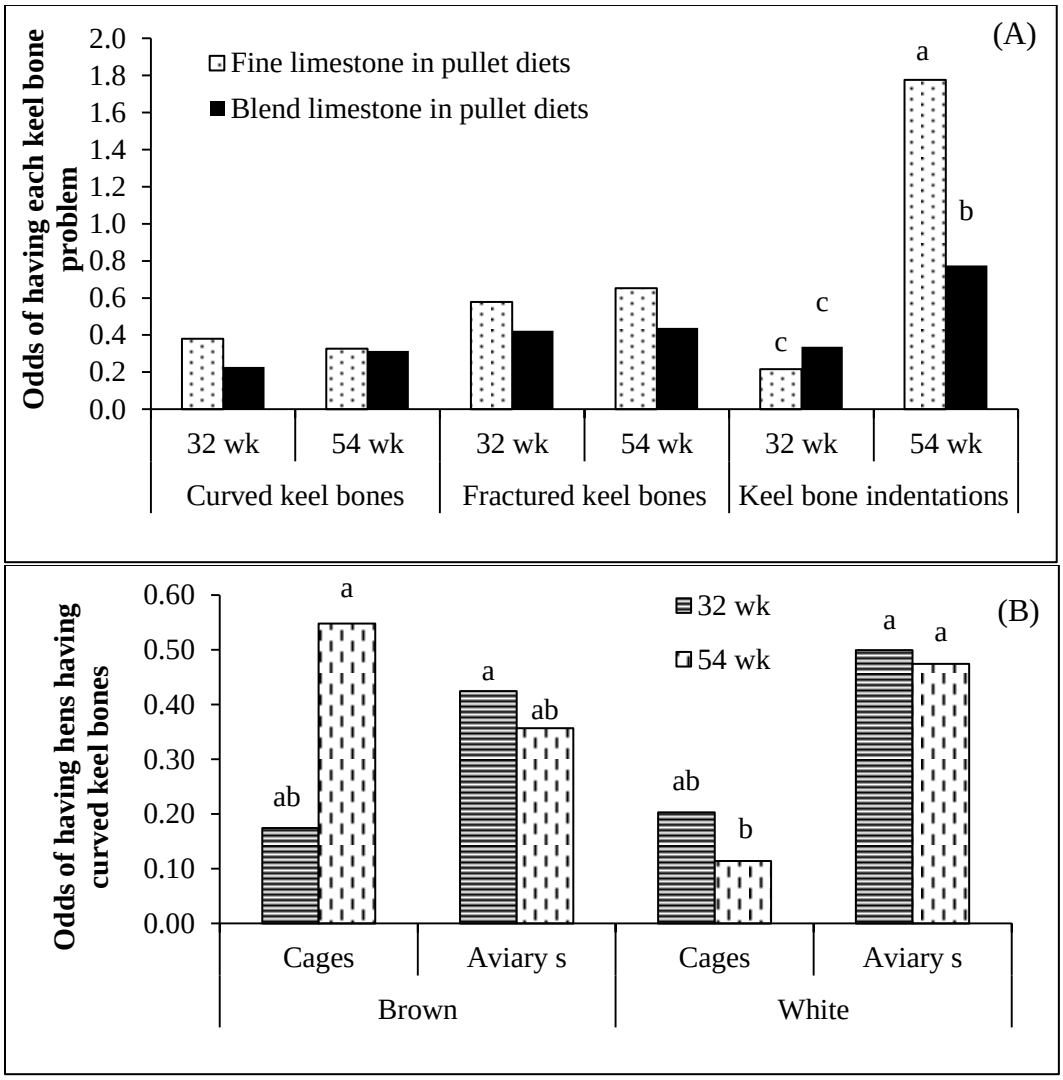

Figure 4.6. Interaction effect of particle size of limestone and age on odds of having curved (P = 0.371), fractured (P = 0.969) and indented (P = 0.023) keel bones (Panel A).Interaction (P = 0.094) effect of housing system, strain and age on odds of having hens with curved keel bones (Panel B).[a-c] Means without a common superscript differ ($P \leq 0.05$).

CHAPTER 5. EFFECT OF PULLET LIMESTONE PARTICLE SIZE ON PERFORMANCE AND BONE HEALTH OF PULLETS AND LAYING HENS RAISED IN DEEP LITTER SYSTEMS

5.1. INTRODUCTION

Pullet nutrition effects on skeletal integrity during the layer phase have not been extensively studied in conventional cage systems and even less so in alternative housing systems. Most past studies evaluating intervention strategies for improving bone integrity in laying hens have been done during the layer phase when the adult hens may already be experiencing osteoporosis. The modern day pullet experiences low feed intake at the onset of lay and has the genetic potential to rise quickly to peak production; adequate body reserves at the onset of production are important to achieve satisfactory hen performance.

One of the major welfare issues facing the egg industry is the high incidence of keel bone fractures and osteoporosis in alternative and traditional housing systems. Some management and nutrition strategies during the pullet phase are being evaluated to improve bone quality of the laying hen. Access to perches during the pullet phase improved bone mineralization at the end of the lay cycle but was not enough to reduce keel bone injuries (Hester et al., 2013). An elevated Ca:P ratio in pullet and pre-lay diets improved femur breaking strength at the end of the layer phase without affecting pullet BW gain and pullet feed efficiency in cage systems (Fosnaught, 2009). Geraldo et al. (2006) reported that hens fed a medium particle size of limestone had higher bone calcium level but similar tibia bone ash at 30 wk of age compared to fine particle size of limestone in pullet diets. There is some evidence that some practices during the pullet phase can have a carry-over effect during the laying cycle.

Our research team found that the inclusion of a limestone blend of fine and large particles improved bone mineral density and decreased curved keel bones compared to fine limestone during the pullet phase (Chapter 3) and reduced overall incidence of keel bone fractures of White hens during the layer phase (Chapter 4). Therefore, the objective of this experiment was to evaluate different limestone particle size fed to White pullets from 9 to 17 wk of age housed in litter floor pens.

5.2. MATERIALS AND METHODS

Birds and husbandry

At 9 wk of age, 120 Bovan White pullets raised in pullet cages were randomly housed in eight floor pens (15 pullets/pen; 929 cm^2/ pullet) divided equally into two separate but identical rooms (4 floor pens/room). Birds were beak-trimmed during the pullet phase. Each floor pen contained a perch, two nest boxes, a feeder tube, and a fountain drinker. Usable perching space and feeder space per bird was 15 and 9 cm, respectively. All procedures were approved by the University of Nebraska-Lincoln Institute of Animal Care and Use Committee.

Pullets were fed a grower (9-12 wk) and a developer (13-15 wk) diet including either a fine (0.431 mm) or a blend of fine and large particles (0.879 mm) of limestone (ILC Resources, Weeping Water, NE). For the pre lay diet (16-17 wk), 75 % of limestone was either fine or blend of fine and large particle of limestone and 25 % of limestone was shell and bone builder (2.486 mm). From 19 to 24 wk of age, hens received the same layer diet containing 65 % of limestone as shell and bone builder and 35 % of fine

limestone. Diets were formulated to have the same nutrient specifications (Table 5.1). All diets were provided in mash form.

Body weight of total population was recorded at 12, 15, 20 and 24 wk of age. Feed intake was determined every two weeks from 10 to 24 wk of age. Egg production was recorded daily from 18 to 24 wk of age. Feed conversion ratio (g feed/g BWG) during the pullet phase was calculated by dividing daily feed intake by average BWG from 9 to 12, and 13 to 15 wk of age. During the layer phase, feed conversion ratio (g feed/g egg) was calculated by dividing daily feed intake by egg mass (egg weight multiply by egg production) from 21 to 23 wk of age. The feed conversion ratio from 16 to 20 wk of age was not calculated because there were few eggs at the start of lay. All productive variables were obtained per pen.

Bone examination

At 13, 16 and 24 wk of age, all pullets were palpated to evaluate keel bone condition. The palpation involved running 2 fingers down the side of the keel bone and feeling for degree of twists (curved keel bones), indentations (depressions with undefined edges) or fractures (sharp edges without palpable callus formation) (Clark et al., 2008; Wilkins et al., 2004).

At 13, 17, 20 and 24 wk of age, a random sample of 5 birds per pen were scanned to determine in-vivo bone mineral density, content and area of right tibias including fibulas using dual-emission x-ray absorptiometry (Norland Medical Systems, Fort Atkinson, WI). Scanned birds were identified by black leg zip ties to take repeated measures using the same bird. Non-anesthetized birds were placed facing up on a foam "bed" device and gently restrained with Velcro straps around the neck, breast including

the wings and shanks for 12 minutes while the scan was taken (Hester et al., 2004). Pullets were individually weighed after the scan to use BW as a covariate in the data analysis.

Egg weight and eggshell characteristics

A total of 10 eggs from each floor pen were sampled to determine eggshell breaking strength at 22, 23, and 24 wk of age. Eggshell breaking strength was tested using a Texture Analyzer (Model TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY) measuring force to break the eggshell in Newton ($Kg \ . \ m/s^2$). Other sample of 10 eggs from each floor pen was weighed, broken open and albumen and yolk were removed to weigh eggshells at 22, 23, and 24 wk of age. Eggshell percentage was expressed as a percentage of initial egg weight. Eggshell mass was calculated by multiplying eggshell weight by egg production of the respective week.

Statistical analysis

A randomized complete block design was used for data analysis considering room as block. Data was analyzed using the GLIMMIX procedure (SAS, Cary, NC). Repeated measures analysis was also used for variables that were measured more than two times to determine changes in data through time. As repeated measures from the same subject are usually dependent, the measurements from the same subject over time might be correlated. To evaluate this correlation structure for each variable the following covariance patterns were tested: 1) compound symmetry, 2) autoregressive of order 1, 3) toeplitz, and 4) unstructured, using the AICC (AIC, Akaike information criterion, with a correction for finite sample sizes) to select the best fit for the model.

There was a total of 8 experimental units resulting in 4 replicates per dietary treatment. Statistical analysis of bone characteristics were conducted with BW at the time of scan as a covariate. BW coefficient of variation and eggshell percentage were subjected to arcsine-square root transformation before statistical analysis. Incidence of each keel bone deformity or injury was analyzed using a binomial logistic regression analysis because this variable was a designation of one of two possible outcomes (binary response), pullets having a specific keel bone issue or pullets having a normal keel bone without any keel bone damage. This analysis resulted in the generation of odds and odds ratio (Szumilas, 2010). Odds (o) are the probability (p) of having a specific keel bone issue over not having it ($1 - p$). Probability of having a specific keel bone issue can be calculated using odds following this formula: $p = o / (1 + o)$. While probabilities range from 0 to 1, odds range from 0 to positive infinity. Means were separated using the LS means function and the SLICE option when applicable. Means were considered different at $P \leq 0.10$.

5.3. RESULTS

There was no main ($P > 0.496$) effect of pullet limestone particle size or interaction with age ($P > 0.490$) (Figure 5.1) for BW, BW coefficient of variation, and BWG during the entire trial. Birds gradually increased BW as they aged ($P < 0.0001$). Also, they had their highest BWG at 12 (12.21 g) wk of age, intermediate BWG at 15 (11.03 g) and 20 (11.24g) wk of age and the lowest BWG at 24 (2.34 g) wk of age ($P < 0.0001$). BW coefficient of variation was not affected by age ($P = 0.333$).

There was no main (P = 0.188) effect of pullet limestone particle size or interaction with age (P = 0.954) (Figure 5.2A) on feed intake. Birds increased their feed intake progressively as they aged (Figure 5.2B) with greater increments from 10 to 12 and 16 to 18 wk of age reaching a peak at 22 wk of age (P < 0.0001). There was no effect of pullet limestone particle size on FCR from 9 to 12 (P = 0.138), 13 to 16 (P = 0.858) and 21 to 23 (P = 0.753) wk of age.

There was no main effect of limestone particle size (P = 0.979) or interaction with age (P = 0.635) (Figure 5.3A) for hen-day egg production. Egg production steadily increased from 18 to 21 wk of age reaching a plateau stage at 22 wk of age (Figure 5.3B) (P < 0.0001).

There was no interaction effect of pullet limestone particle size and age on egg weight (P > 0.10), or any eggshell characteristics (P > 0.10) (Table 5.2). There was no main effect of pullet limestone particle size on overall egg weight (P = 0.581), overall eggshell weight (P = 0.352), overall eggshell mass (P = 0.971) and overall eggshell percentage (P = 0.564). Hens fed a limestone blend of fine and large particles in pullet diets had greater eggshell breaking strength than hens fed a fine limestone in pullet diets (P = 0.072). Regardless of limestone particle size, egg weight (P = 0.001) and eggshell percentage (P < 0.0001) were the only egg characteristics affected by age from 22 to 24 wk of age. The lowest egg weight and the highest eggshell percentage were observed at 22 wk of age.

Bone characteristics are shown in Table 5.3. Pullets fed a limestone blend of fine and large particles had greater BMD than pullets fed fine limestone only at 17 wk of age (P = 0.102). Limestone particle size did not affect bone mineral content and bone area of

tibias at any other age ($P \geq 0.121$). Pullet limestone particle size did not affect overall curved ($P = 0.154$), indented ($P = 0.200$) or fractured ($P = 0.249$) keel bones (Table 5.4).

The odds of having normal keel bones progressively decreased as the hens aged ($P = 0.0004$). Older hens had the highest odds of having curved keel bones compared to those birds at younger ages ($P = 0.006$). Also, there an age effect ($P = 0.030$) on odds of having keel bone indentations indicating that 24-wk old hens had higher odds of having keel bone indentations than hens at 13 wk ($P = 0.011$) and at 16 wk of age ($P = 0.091$) whereas odds of having keel bone indentations remained fairly constant from 13 to 16 wk of age ($P = 0.205$).

5.4. DISCUSSION AND CONCLUSIONS

The use of pullet limestone particle size did not affect BW, BWG and BW coefficient of variation during the pullet and layer phase. The lack of effect of pullet limestone particle size on hen day egg production was also observed in a similar study for White hens in aviary systems (Chapter 4). The fact that only eggshell strength but not eggshell percentage was affected by pullet limestone particle size suggests that other factors such as eggshell thickness, inorganic microstructure or even quality of membranes and organic matrix could have exerted changes in eggshell breaking strength (Bain, 2004). Further studies of eggshell quality variables are necessary to investigate how pullet limestone particle size affects eggshell breaking strength during the peaking egg production phase.

Although hens fed a limestone blend of fine and large particles in pullet diets had greater bone mineral density during the onset of lay, the use of a limestone blend did not

significantly affect incidence of specific keel bone deformity or fractures. In a previous similar experiment (Chapter 3) in aviary systems, the use of blend rather than fine limestone reduced incidence of curved keel bones at 16 wk of age. In this experiment, there is a numerical trend showing similar responses. Probably, the lack of pullet limestone particle size effect on keel bone condition might be a result of a less complex environment in the deep litter system reducing risk of injuries compared to the aviary system which had three metal tiers and perches in multiple levels (Chapter 4).

In summary, the use of a blend of limestone particle size during the pullet rearing increased bone mineral density at 17 wk of age and improved overall eggshell strength. The lack of other major effects of pullet limestone particle size could be because of later provision of dietary treatments during the pullet phase, smaller group size, and lower feed competition compared with previous studies (Chapter 3 and 4).

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Table 5.1. Diet composition and nutrient contents (as-fed basis)

Ingredient (%)	Grower (9-12 wk)	Developer (13-15 wk)	Pre lay (16-17 wk)	Layer (18-24 wk)
Corn	66.11	70.00	61.64	47.61
Soybean meal	24.18	20.36	20.88	28.72
DDGS	5.00	5.00	5.00	5.00
Vegetable oil	0.53	0.30	2.85	5.09
Dicalcium phosphate	1.54	1.35	1.36	1.56
Limestone[1]	1.43	1.89	7.18	11.08
Salt	0.38	0.37	0.39	0.43
DL-Methionine	0.15	0.11	0.11	0.19
L-Lysine HCl	0.13	0.12	0.05	0.00
L-Threonine	0.04	0.00	0.00	0.00
Vitamin-Mineral Premix[2]	0.50	0.50	0.50	0.30
Calculated nutrients				
Metabolizable energy, kcal/kg	2970.00	2975.00	2935.00	2875.00
Crude protein, %	18.00	16.45	16.00	18.60
Lysine , %	1.00	0.89	0.82	0.96
Methionine, %	0.42	0.36	0.36	0.47
Methionine+Cysteine, %	0.73	0.65	0.64	0.78
Threonine, %	0.71	0.62	0.60	0.71
Tryptophan, %	0.22	0.19	0.19	0.24
Calcium, %	1.00	1.10	2.85	4.20
Total analyzed calcium[3], %	1.06 / 1.25	1.45 / 1.37	2.95/ 2.84	4.53
Available phosphorus, %	0.50	0.46	0.46	0.50
Total analyzed phosphorus[3], %	0.73 / 0.80	0.79 / 0.73	0.81 / 0.76	0.69
Sodium, %	0.18	0.17	0.18	0.19

Ronozyme (500 FTU/g) was considered to release 0.1 % of Ca and P.

[1] ILC Resources, Weeping Water, NE. 100 % of fine (0.341 mm) or blend (0.891 mm) limestone was provided for grower and developer diets. For pre-lay diets, 75 % of limestone was either fine (0.431 mm) or blend (0.891 mm) and 25 % of limestone was shell and bone builder (2.486 mm). For layer diets, 65 % of limestone was provided as shell and bone builder (2.486 mm) and 35 % of fine limestone (0.431 mm).

[2]Vitamin and trace minerals provided the following per kilogram of feed: Vitamin A (retinyl acetate, 10,788 IU); vitamin D_3 (cholecalciferol, 4,381 IU); vitamin E (DL-α-tocopheryl acetate, 32 IU); vitamin K_3 (menadione dimethpyrimidinol, 4.0 mg); vitamin B_2 (riboflavin, 7.0 mg); vitamin B_5 (pantothenic acid 9.0 mg); Vitamin B_3 (niacin, 46 mg); vitamin B7 (biotin, 93 mg); vitamin B_{12} (cobalamin, 11 mg); and choline ($C_5H_{14}ClNO$, 682 mg). Mn (MnO, 100 mg); Cu ($CuSO_4H_2O$, 7.5 mg); Fe ($FeSO_4H_2O$, 32 mg); Zn (ZnO, 73 mg); and Se (Na_2SeO_3, 0.24 mg).

[3] Total content of Fine limestone-Diet/Total content of blend limestone-Diet. Method: AOAC 985.01: wet ash procedure that required mineral acids and heat.

Table 5.2. Effect of particle size of limestone in pullet diets (LPS) on egg weight and weight, percentage, mass, breaking strength of eggshells

Limestone particle size	Age (wk)	Egg W (g)	Eggshell W (g)	Eggshell mass (g/bird/d)	Eggshell (%)	Eggshell Strength (N)
Fine		52.77	6.66	5.86	12.63	64.39^{B}
Blend		52.52	6.60	5.85	12.57	66.73^{A}
		(0.32)	(0.04)	(0.13)	(0.07)	(0.92)
	22	51.24^{b}	6.67	5.70	13.01^{a}	65.98
	23	53.68^{a}	6.67	6.04	12.43^{b}	66.92
	24	53.03^{a}	6.55	5.83	12.35^{b}	63.78
		(0.39)	(0.06)	(0.15)	(0.08)	(1.12)
Fine	22	50.87	6.61	5.59	13.00	64.45
	23	53.75	6.71	5.91	12.48	65.37
	24	53.70	6.66	6.08	12.40	63.34
Blend	22	51.60	6.72	5.80	13.03	67.51
	23	53.61	6.64	6.17	12.39	68.47
	24	52.35	6.44	5.59	12.31	64.22
		(0.55)	(0.08)	(0.22)	(0.12)	(1.59)
Source of variation				*P*-values		
Limestone particle size		0.581	0.352	0.971	0.564	0.072
Age		0.001	0.280	0.318	< 0.0001	0.130
LPS×Age		0.192	0.176	0.194	0.838	0.728

^{a,b} Means within a column lacking a common superscript differ ($P \leq 0.05$). ^{A,B} Means within a column lacking a common superscript differ ($P \leq 0.10$). Values within parentheses are SEM.

Table 5.3. Effect of pullet limestone particle size on bone characteristics.

Limestone particle size	13 wk	17 wk	20 wk	24 wk
Bone mineral density (g/cm^2)				
Fine	0.178	0.219^{B}	0.271	0.257
Blend	0.177	0.228^{A}	0.278	0.259
	(0.004)	(0.003)	(0.004)	(0.007)
Bone mineral content (g)				
Fine	1.77	2.40	3.10	2.98
Blend	1.72	2.44	3.13	2.95
	(0.02)	(0.11)	(0.05)	(0.13)
Bone area (cm^2)				
Fine	9.97	10.96	11.44	11.59
Blend	9.76	10.69	11.32	11.38
	(0.23)	(0.47)	(0.18)	(0.31)
Source of variation		*P*-values		
Bone mineral density				
Limestone particle size	0.875	0.102	0.345	0.859
BW	0.714	0.001	0.082	0.243
Bone mineral content				
Limestone particle size	0.121	0.833	0.715	0.878
BW	0.031	0.048	0.392	0.380
Bone area				
Limestone particle size	0.558	0.715	0.650	0.669
BW	0.111	0.494	0.290	0.761

$^{a-c}$ Means within a column lacking a common superscript differ ($P \leq 0.05$). ^{A,B} Means within a column lacking a common superscript differ ($P \leq 0.10$).Values within parentheses are SEM.

Table 5.4. Effect of particle size of limestone in pullet diets on probabilities of observing birds having curved, indented, or fractured keel bones

Limestone particle size	Age (wk)	Curved keel bones		Keel bone indentations		Keel bone fractures[1]	
		Mean	CI[4]	Mean	CI[4]	Mean	CI[4]
				Odds[2]			
Fine		0.18	0.10-0.32	0.43	0.26-0.70	0.13	0.06-0.31
Blend		0.08	0.03-0.22	0.27	0.15-0.49	0.06	0.02-0.21
	13	0.04[b]	0.01-0.15	0.19[b]	0.10-0.39	-	-
	16	0.12[b]	0.06-0.24	0.33[ab]	0.19-0.58	0.06	0.02-0.19
	24	0.36[a]	0.22-0.59	0.63[a]	0.38-1.04	0.15	0.07-0.33
Treatment comparisons				Odd ratios[3]			
Fine vs. Blend		2.19	0.68-7.06	1.57	0.73-3.38	2.16	0.49-0.45
	13 vs. 16	0.32	0.07-1.43	0.58	0.24-1.41	-	-
	13 vs. 24	0.11	0.03-0.44	0.31	0.13-0.72	-	-
	16 vs. 24	0.34	0.14-0.79	0.53	0.25-1.13	0.37	0.09-1.63

Source of variation	p-values		
Limestone particle size	0.154	0.200	0.249
Age	0.006	0.030	0.152
Limestone particle size×Age	0.559	0.418	0.751

[a,b] Means within a column lacking a common superscript differ ($P \leq 0.05$). [1] Keel bone fractures only correspond to 16 and 24 wk of age because there were any keel bone fractures at 13 wk of age. [2] Odds of having a specific keel bone problem over not having it for each treatment group.[3] Ratio of the odds of having specific keel bone problem for two treatment groups. [4] Confidence interval.

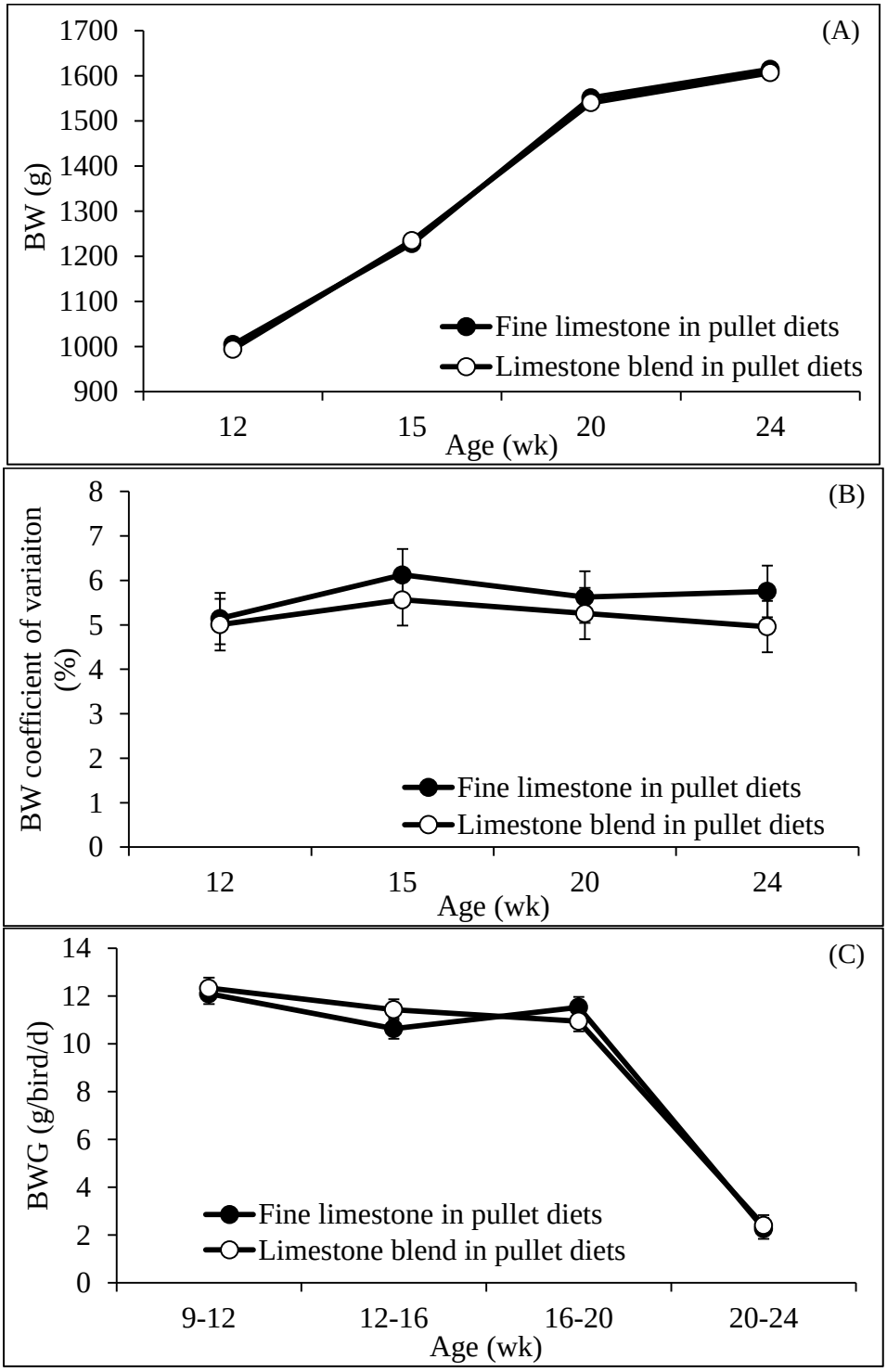

Figure 5.1. Effect of particle size of limestone in pullet diets over time on BW (*P* = 0.806) (Panel A), BWG (*P* = 0.878) (Panel B) and coefficient of variation of BW (*P* = 0.490) (Panel C). Bars represent SEM.

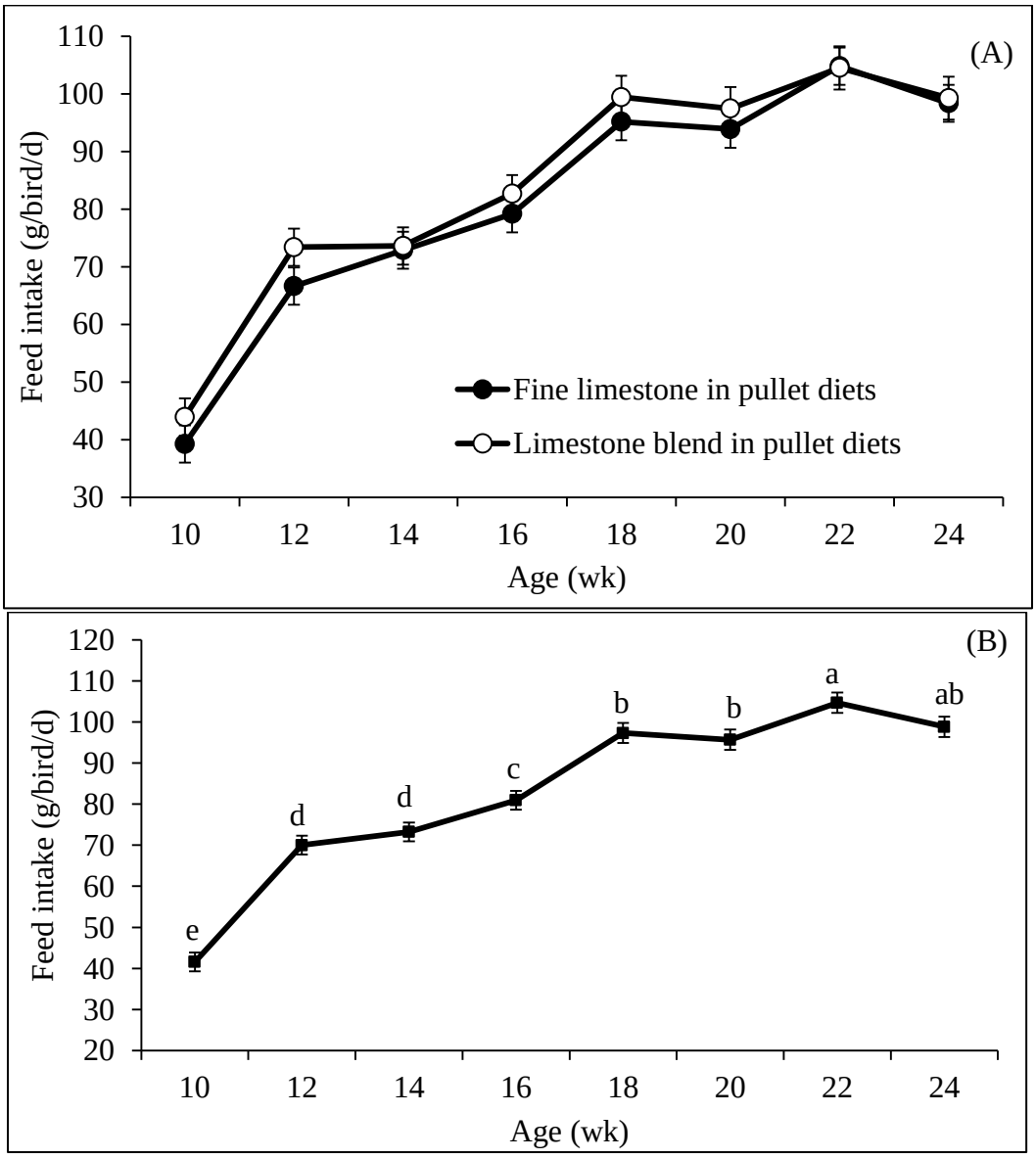

Figure 5.2. Effect of particle size of limestone in pullet diets over time ($P = 0.954$) (Panel A) and main effect of age ($P < 0.0001$) (Panel B) on feed intake. [a-e] Means lacking a common superscript differ ($P \leq 0.05$). Bar represent SEM.

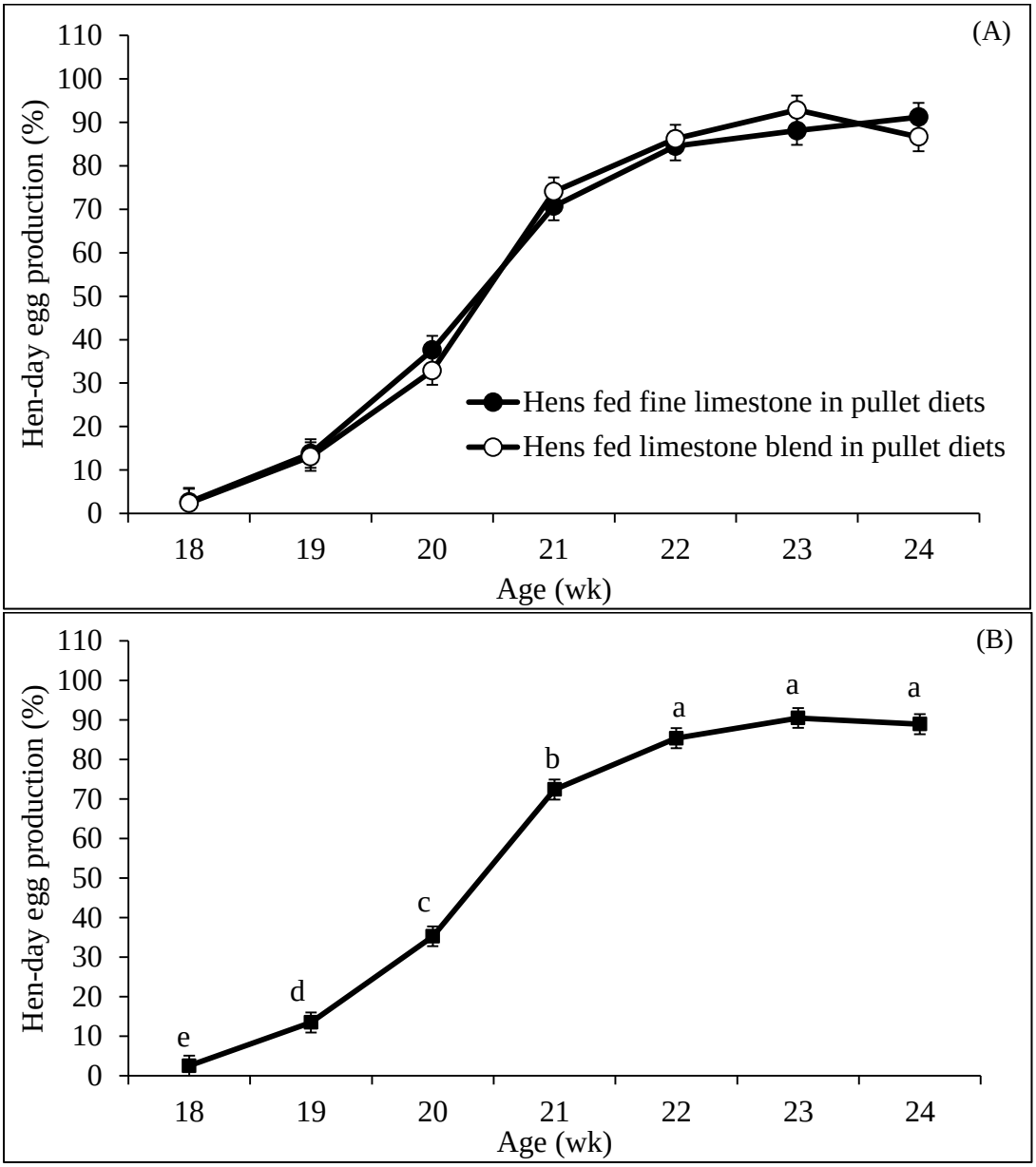

Figure 5.3. Effect of particle size of limestone in pullet diets over time ($P = 0.635$) (Panel A) and effect of age ($P < 0.0001$) (Panel B) on hen day egg production. [a-e] Means lacking a common superscript differ ($P \leq 0.05$). Bar represent SEM.

CHAPTER 6. THE EFFECTS OF TWO GENETIC LINES ON SPATIAL DISTRIBUTION AND USAGE AND PREFERENCE OF NEST AND PERCH IN AVIARY SYSTEMS

6.1. INTRODUCTION

Genetic selection for hen adaptation to alternative housing systems is important to choose the most appropriate strain for specific environmental conditions in alternative housing systems. Adaptation to nests, drinker nipples, and perches among others are important for hen's welfare, production and health. Hen adaptation to alternative housing systems has been evaluated by the study of use of vertical levels (Channing et al., 2001; Oden et al., 2002), egg laying location, egg production and mortality (Van Horne, 1996; Colson et al., 2008).

Hens are highly motivated to use perches and will use them to reach resources, to roost at night, and to escape unwanted attention from other birds (Sandilands et al., 2009). Several researchers have stated that perches may improve welfare by reducing incidence of feather pecking, cannibalism and even aggression (Sandilands et al., 2009).Furthermore, high use of nest boxes indicates that laying hens place considerable value on laying eggs in a secluded area (Cooper and Albentosa, 2003; Blokhuis, 2007).

Some experiments in floor pens or colony cages have shown that Brown and White Leghorn hens have different behavior and usage of resources (Faure and Jones, 1982; Silversides et al., 2012; Abrahamsson and Tauson, 1995). Sandilands et al. (2009) suggested that the ability of Brown hens to perch might be compromised by their higher body weight to wing area ratio compared to White Leghorn hens.

The acceptance of multilevel aviary systems is increasing, as it diversifies the hen's behavior repertory and allows producers to accommodate a larger number of hens. However, there is no study of strain effect on the hen adaption to the aviary system as indicated by the ability to utilize resources. Therefore, the objective of this trial was to evaluate spatial distribution, nest and perch usage and preference during the layer phase of Brown and White Leghorn hens raised in floor pens with perches.

6.2. MATERIAL AND METHODS

At 5 wk of age, 400 floor raised pullets, Lohmann Brown and Bovan White Leghorn strains, in equal numbers, were placed into 8 aviary units (25 Brown and 25 White hens/aviary unit). Pullets had not been beak trimmed. Design of aviary system (Natura 60, Big Dutchman Inc.) is shown in Figure 6.1. Each aviary unit had 3 metal sloped tiers and an indoor litter area (677 cm^2/bird) underneath and beyond the aviaries. A manure belt system under the bottom tier was used for manure removal every three d. Floor litter was changed or supplemented as required to maintain it in a dry condition. Sloped stairways allowed birds to move freely among the different levels in the aviary. Hens had access to the litter area throughout the day. A sloped nest area (120 cm^2/bird) lined with brown artificial turf was located at the top tier in which eggs rolled out to an egg collection area in the inspection aisle. External feeder troughs (4.8 cm/bird) were provided in the middle and lower tier. Internal feeder troughs were not used in this trial. Six and two nipple drinkers were provided in the top and bottom tier, respectively. Hens were provided with two perches in the top tier, four perches in the middle tier, and four perches in the lower tier (22 cm/bird). Feed and water was provided ad libitum. Feeding

and egg collection was done manually once daily at 9:00 AM. Lights went on at 7:00 AM and off at 10:00 PM to provide 15 hr of light.

At 25 wk of age, the number of birds on lower, middle, upper tier and litter area and perches from each aviary unit was recorded by scan sampling every four hours from 8:00 AM to 12:00 AM to evaluate spatial distribution and perch preference. Total number of birds perching in each aviary unit was recorded every four hours from 8:00 AM to 12:00 AM at 15, 25 and 35 wk of age to evaluate perch usage. Aviary units were scanned in a random order. Number of eggs laid in the nest area, metal tiers, and litter floor eggs from each aviary unit were recorded daily from 22 to 53 wk of age to evaluate nest usage and egg location in the aviary system. All procedures were approved by the University of Nebraska-Lincoln Institute of Animal Care and Use Committee.

A split plot factorial design with Poisson distribution was used for analysis of spatial distribution and perch preference. Strains were considered as subplots and location as main factor, and time of day as repeated measures. Poisson distribution was implemented to evaluate the rate of occurrence of an event estimated by relating the logarithmic transformation of predicted value to a linear function (Petrie and Watson, 2013). The relative rate represents the ratio of rates between two treatment groups (Petrie and Watson, 2013).

Nest and perch usage were analyzed using a binomial logistic regression analysis because this variable was a designation of one of two possible outcomes (binary response), birds nesting or perching or birds not performing these activities. This analysis resulted in the generation of odds and odds ratio (Szumilas, 2010). Odds (o) are the probability (p) of birds nesting or perching over not doing these activities ($1 - p$).

Probability of birds nesting or perching can be calculated using odds following this formula: $p = o / (1 + o)$. While probabilities range from 0 to 1, odds range from 0 to positive infinity. Strain was considered subplot, and time of day and age were considered repeated measures.

As repeated measures from the same subject are usually dependent, the measurements from the same subject over time might be correlated. To evaluate this correlation structure for each variable the following covariance patterns were tested: 1) compound symmetry, 2) autoregressive of order 1, 3) toeplitz, and 4) unstructured, using the AICC (AIC, Akaike information criterion, with a correction for finite sample sizes) to select the best fit for the model. There were a total of 8 replicates for each treatment combination. Mean were considered different at P-value ≤ 0.10.

6.3. RESULTS AND DISCUSSION

Spatial distribution

There was an interaction effect of genetic strain, time of day, and location ($P < 0.0001$) (Table 6.1) on spatial distribution in aviary systems at 25 wk of age. During the morning and early afternoon, a higher number of White hens than Brown hens were observed at the top tier ($P < 0.05$) and hens from both strains seemed evenly spread out for the other areas ($P > 0.10$) (Figure 6.2). During late afternoon and night, a higher number of White hens were observed in the middle and top tier while a higher number of Brown hens were observed in the litter area and bottom tier. At 8:00 AM, hens from both strains were observed with the highest number in the litter area compared to any other aviary area; this continued to be true only for White hens until 12:00 PM.

Similar to our results, Abrahamsson (1995) reported higher a number of Brown hens in the litter area than White hens in a three-tier aviary system. A possible contributory factor of the strain differences could be the different profile BW of these two genetic lines. White hens (with lower BW than Brown hens) have lower wing loading, facilitating movement vertically throughout the aviary system. Furthermore, because bottom tier and the litter area provided almost all resources such as feed, water, perch and scratching area, the expected necessity of Brown hens to move to upper tiers for nesting might have not been high enough.

Perch usage and preference

There was an interaction ($P = 0.024$) among strain, age and time of day on perch usage (Table 6.2, Figure 6.2). The odds of observing White hens perching were higher than the odds of observing Brown hens perching during late morning, late afternoon and night at 15 wk of age, from early afternoon to night at 25 wk of age, and during all afternoon at 35 wk of age. Also, there was an interaction ($P = 0.001$) between strain and time of day indicating similar strain effects on overall perch usage regardless of age (Figure 6.4) indicating that the odds of observing White hens perching was higher than the odds of observing Brown hens perching during the entire day with the exception of 8:00 AM.

There was an interaction ($P = 0.007$) effect among strain, time of day and perch location on predicted number of hens perching (Table 6.1, Figure 6.5). At 8:00 AM, a higher number of Brown hens were perching in the lower and middle tier compared to White hens. At 12:00 PM and 8:00 PM, a higher number of Brown hens were perching in the lower tier than White hens whereas a higher number of White hens were perching in

the top tier than Brown hens. At 4:00 PM, a higher number of White hens were perching in the top tier than Brown hens. At 12:00 AM, a higher number of Brown hens were perching in the lower tier while a higher number of White hens were perching in the middle and top tier.

Silversides et al. (2012) found more White hens (76.3%) used perches than Brown hens (6.8 %) in floor pens right before light went off. Faure and Jones (1982) also reported that Brown Leghorn hens almost completely failed to use high perches and had lower perch usage than White Leghorn in litter floor pens. In the present study, the preference of perches in lower tiers of Brown hens could be a result from a less capacity to move vertically throughout the aviary system. On the other hand, White hens seemed to prefer perches in higher tiers especially during the night after light were off. In a study comparing various perch heights, during the daytime lower perches were used more for standing and walking, while higher perches were used more for sitting and sleeping (Struelens et al., 2008).

Nest usage and egg location

White hens had higher nest usage than Brown hens expressed by the higher odds of observing eggs from the nest area ($P = 0.071$) (Table 6.3). There was no interaction effect of age and strain ($P = 0.999$) indicating that the effect of strain on nest usage more fairly consistent during the entire trial. There was a strain and egg location interaction ($P < 0.0001$) for number of laid eggs indicating that White hens were laying more eggs in nest area and litter floor area compared to Brown hens whereas White hens were laying fewer eggs in the metal aviary tiers in comparison with Brown hens (Table 6.4).

In accordance with our results, White hens laid most of their eggs in nest boxes, whereas Brown hens laid half of their eggs on the floor in a deep litter system (Singh et al., 2009). Also, Abrahamsson (1995) reported observing higher number of White hens in the nest area than Brown hens in a three-tier aviary system. In the current study, higher preference of nest area of White hens than Brown hens might be a result from a greater ability to reach the top tier a greater motivation to lay in higher levels. The fact that most mislaid eggs were laid on metal tiers for Brown hens but on litter floor for White hens suggested that these strains have different broody behaviors. In either area, mislaid eggs are more likely to be dirty and cracked resulting in economic losses.

6.4. CONCLUSIONS

White hens showed greater degree of adaptation to aviary systems than Brown hens expressed by greater usage of perch and nest and elevated tiers. White hens seemed to be more suitable for aviary systems than Brown hens in terms of utilization of resources. However, the higher activity and movement throughout the aviary of White hens could also potentially incur in greater incidence of bone fractures. Further studies of nest design to increase attractiveness for Brown hens in aviary systems are necessary to prevent mislaid eggs.

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Table 6.1. Source of variation and *P*-values of perch preference and spatial distribution

Source of variation	Spatial distribution	Perch preference
Strain	0.052	0.007
Location[1]	< 0.0001	0.213
Time of day	0.007	< 0.0001
Strain×Location	< 0.0001	< 0.0001
Time of day×Location	< 0.0001	0.677
Strain×time of day	0.133	0.018
Strain×time of day×Location	< 0.0001	0.036

[1]For spatial distribution, number of hens in each of the three tiers and litter area were taken into account for analysis. For perch preference, hens roosting in perches located in each of the three tier tiers were taken into account for analysis.

Table 6.2. Effect of strain and age on perch usage in aviary systems

Age (wk)	Strain	Mean	Confidence interval
		Odds[1]	
	Brown	0.41[b]	0.33 - 0.50
	White	0.74[a]	0.61 - 0.89
15		0.82[a]	0.70 - 0.96
25		0.41[b]	0.34 - 0.50
35		0.50[b]	0.41- 0.59
Treatment comparisons		Odds ratio[2]	
	Brown vs. White	0.56	0.43 - 0.74
15 vs. 25		1.99	1.64 - 2.42
15 vs. 35		1.65	1.36 - 2.01
25 vs. 35		0.83	0.67 - 1.02
Source of variation		*P*-values	
Strain		0.0004	
Time of day		< 0.0001	
Strain×Time of day		0.0002	
Age		< 0.0001	
Age×Strain		0.590	
Age×Time of day		0.180	
Age×Strain×Time of day		0.013	

[1] Odds of observing hens using perches over not observing them for each treatment group.
[2] Ratio of the odds of observing hens using perches for two treatment groups.
[a-b] Means within a column lacking a common superscript differ ($P \leq 0.05$).

Table 6.3. Effect of strain on nest usage in a three-tier aviary system

Strain	Mean	Confidence interval
	Odds[1]	
Brown	2.13	1.79 - 2.54
White	2.65	2.24 - 3.15
Treatment comparison	Odds ratio[2]	
Brown vs. White	0.80	0.63 - 1.03
Source of variation	*P*-values	
Strain	0.071	
Age	0.982	
Strain×Age	0.999	

[1] Odds of observing eggs in nest area over not observing them for each treatment group.
[2] Ratio of the odds of observing eggs in nest area for two treatment groups.

Table 6.4. Effect of strain and nest location on number of laid eggs and relative rates

Strain	Egg location	Mean	Confidence interval
		Predicted number of laid eggs (aviary unit/wk)	
Brown	Nest	79.22[b]	74.21 - 84.50
	Wire	16.28[d]	14.07 - 18.84
	Floor	19.22[d]	16.83 - 21.96
White	Nest	97.73[a]	92.24 - 103.54
	Wire	1.32[e]	0.77 - 2.28
	Floor	34.95[c]	31.69 - 38.54
Treatment comparisons			
		Relative rate[1]	
Brown	Floor vs. Nest	0.24	0.21 - 0.28
	Floor vs. Wire	1.18	0.98 - 1.42
	Nest vs. Wire	4.87	4.19 - 5.66
White	Floor vs. Nest	0.36	0.32 - 0.40
	Floor vs. Wire	26.46	15.45 - 45.31
	Nest vs. Wire	73.97	43.19 - 126.72
Brown vs. White	Floor	0.55	0.47 - 0.64
Brown vs. White	Nest	0.81	0.75 - 0.88
Brown vs. White	Wire	12.32	7.17 - 21.19

Source of variation	*P*-values
Strain	0.0003
Egg location	< 0.0001
Strain×Egg location	< 0.0001
Age	0.334
Strain×Age	0.999
Egg location×Age	0.308
Strain×Egg location×Age	0.822

a-e Means within a column lacking a common superscript differ ($P \leq 0.05$).
[1]Ratio between the rates of two treatment groups.

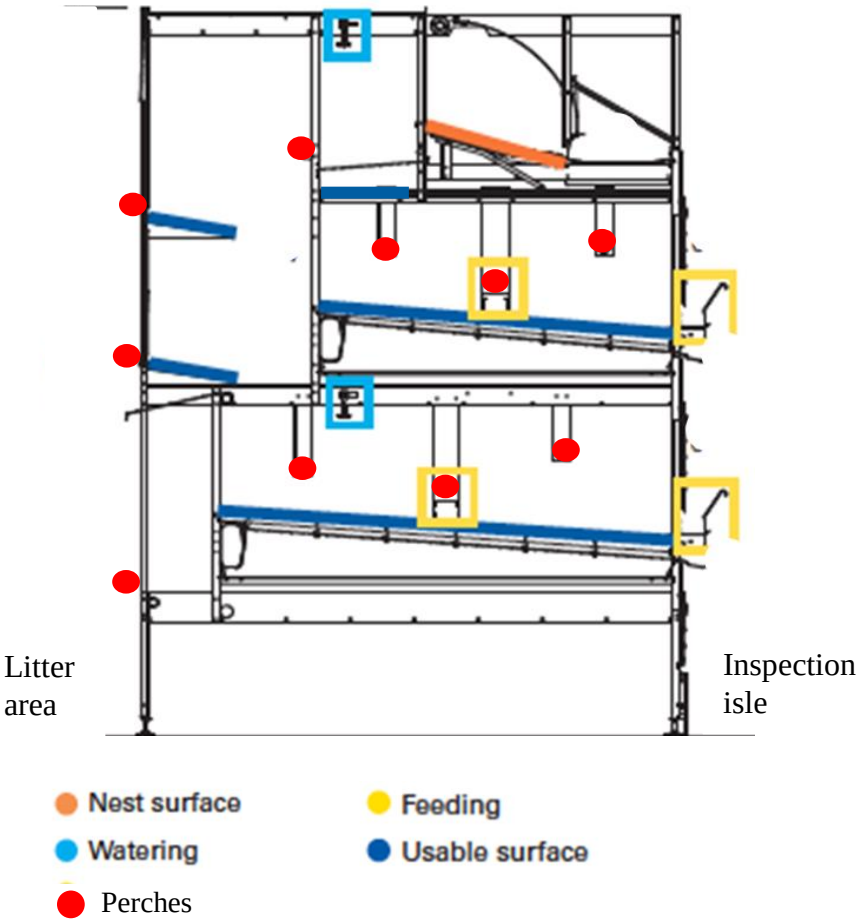

Figure 6.1. Design of aviary system

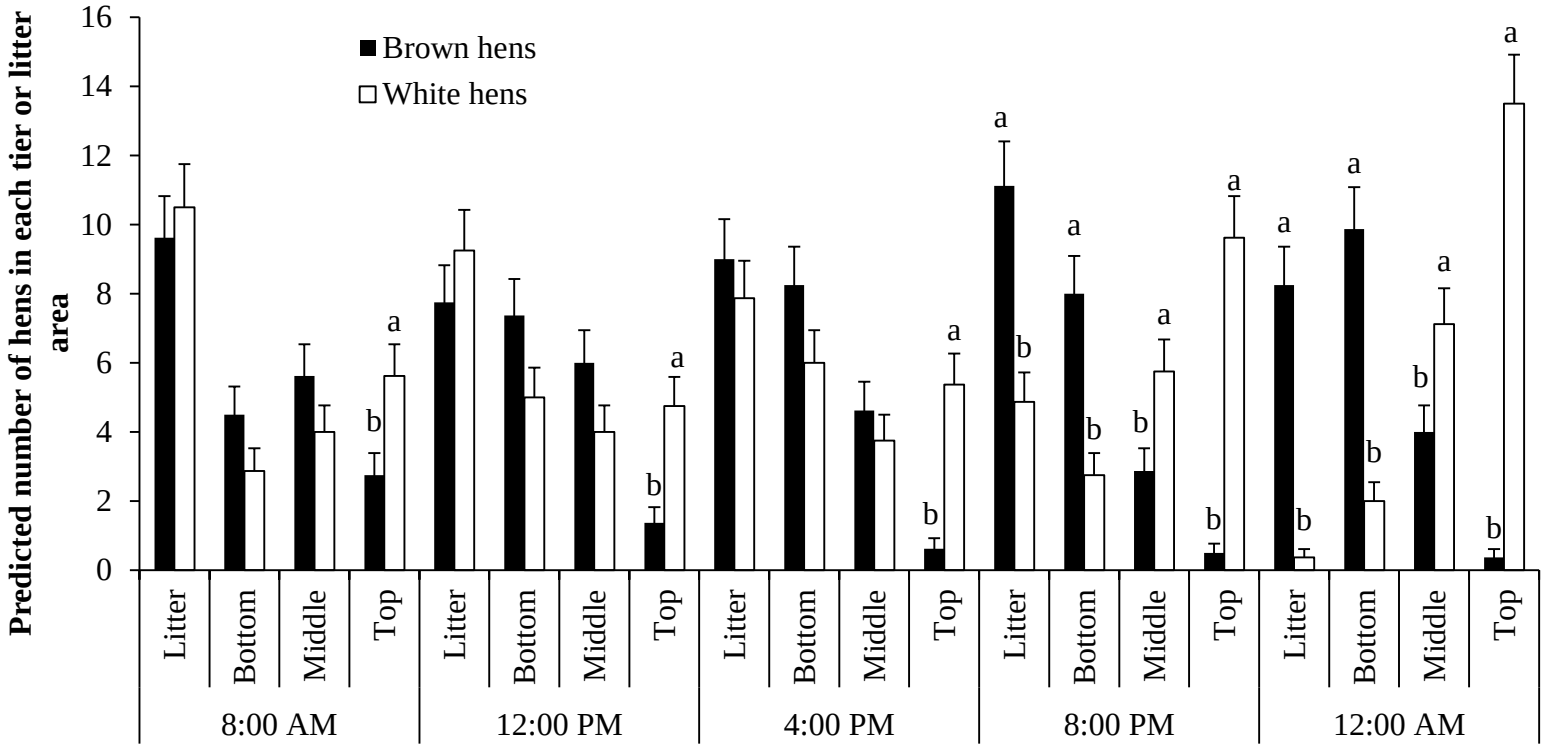

Figure 6.2. Effect of strain on spatial distribution in three-tier aviary system with access to indoor litter area at 25 wk of age. a-b Means within a time of day and a location lacking a common superscript differ ($P \leq 0.05$). Average number of hens from each strain in each aviary unit was 23. Bars represent SEM.

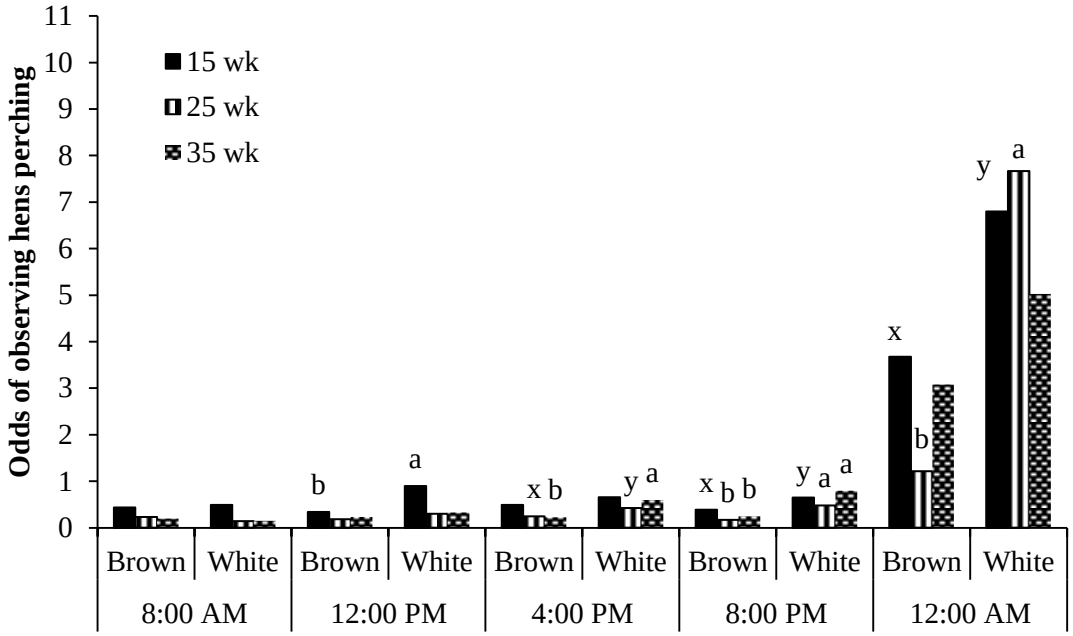

Figure 6.3. Interaction effect of strain, age and time of day on odds of observing hens perching. [a,b] Means within an age and time of day lacking a common superscript differ ($P \leq 0.05$). [x, y] Means within an age and time of day lacking a common superscript differ ($P \leq 0.10$).

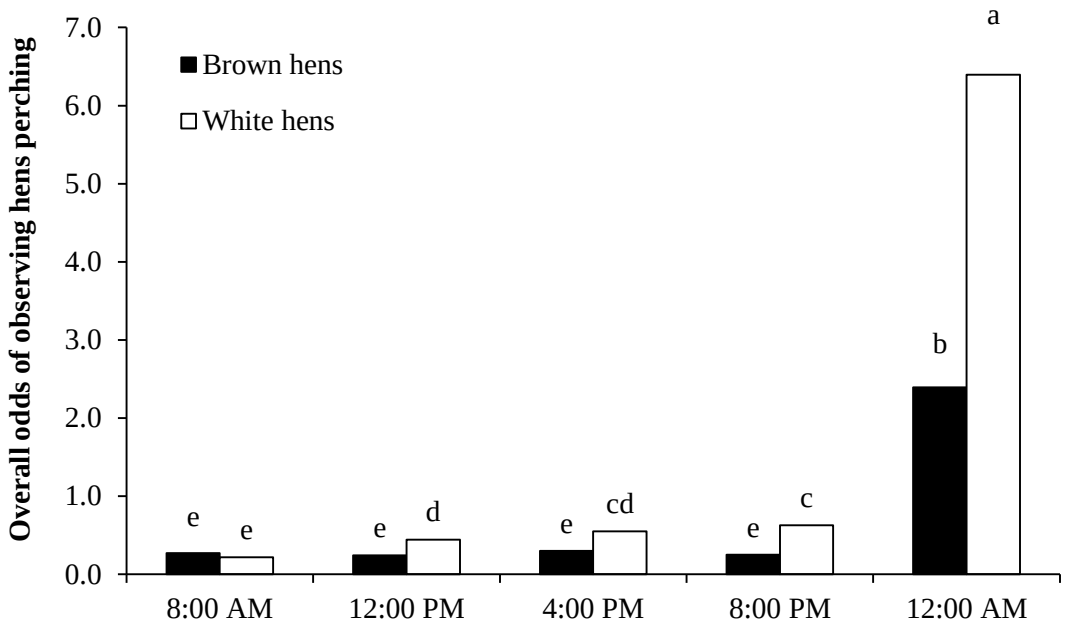

Figure 6.4. Effect of strain and time of day on overall odds of observing hens perching. [a-e] Means lacking a common superscript differ ($P \leq 0.05$). Bars represent SEM.

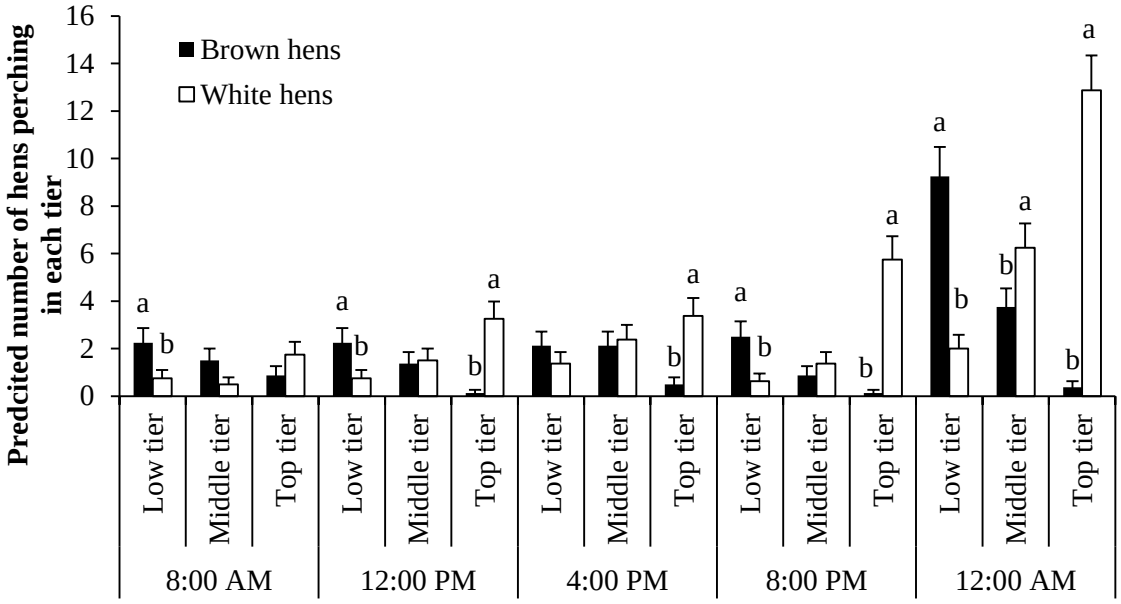

Figure 6.5. Effect of strain on perch preference in three-tier aviary system at 25 wk of age. [a-b] Means within a time of day and a tier lacking a common superscript differ ($P \leq 0.05$). Bars represent SEM.

CHAPTER 7. SUMMARY AND IMPLICATIONS

The use of the blended limestone (0.891 mm) rather than the fine limestone (0.431 mm) during the pullet phase improved bone mineral density at the onset of lay in Study 3 (aviaries and cages) and in Study 5 (floor pens). As during this time most pullets were undergoing sexual maturity, it is possible that the increased tibia BMD indicated improvement in mineralization of medullary bone, a labile calcium reservoir for eggshell production. In fact, hens fed the blended limestone as pullets had increased eggshell percentage for Brown cage hens and increased overall eggshell weight in Study 4 (aviaries and cages) and increased eggshell breaking strength in Study 5 (floor pens).

The use of the blended limestone decreased keel bone curvatures at the end of the pullet phase (Study 3) and reduced keel bone indentations at the end of the layer phase (Study 4). Although keel bone integrity improved in 54-wk-old hens fed the limestone blend as pullets, overall tibia BMD at 52 wk of age was not affected by limestone particle size. In fact, White hens fed limestone blend as pullets had lower tibia BMD. These results suggested that other factors rather than BMD such as bone microstructure (porosity) or organic matrix were involved in keel bone quality late during lay cycle.

The provision of the blended limestone reduced overall incidence of keel bone fractures for only White hens (Study 4). Higher use of perches and nest areas by White strain may have increased the potential risk of keel bone fractures compared to Brown hens (Study 6). In contrast, in Study 5, the utilization of the blended limestone did not affect keel bone integrity perhaps because floor pens provided less complex environment than the multi-tier aviary systems used in Study 4, reducing the potential risk of keel bone damage. Also, it is possible that the later start of the provision of the blended limestone in

Study 5 (9 wk of age) compared to Study 3 - 4 (7 wk of age) might have affected keel bone integrity response.

Thus, the use of the limestone blend rather than fine limestone in pullet diets has promising positive effects on keel bone integrity late in the lay cycle in alternative housing systems for White Leghorn hens. Further investigation is needed to evaluate limestone particle size for Brown pullets raised in aviary systems because the use of the blended limestone in pullet diets reduced egg production for Brown aviary hens (Study 4).

The exact age during which the blended limestone should be incorporated in the pullet diet still needs further investigation. Examination of medullary bone properties during sexual maturity and quality of organic matrix of the keel bone might be useful evaluations to clarify how limestone particle size during the pullet phase affected bone quality during the layer phase.